ANTITHYROID AND ANTIOXIDANT EFFECTS OF *Boerhaavia diffusa* LINN ON THE L-THYROXINE INDUCED HYPERTHYROIDISM IN RATS

Dissertation submitted to
THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY, CHENNAI.
In partial fulfillment of the requirement for the award of the degree of

MASTER OF PHARMACY
IN

PHARMACOLOGY

By

(Reg No: 261325351)

Under the Guidance of

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ARULMIGU KALASALINGAM COLLEGE OF PHARMACY,
ANAND NAGAR, KRISHNANKOIL – 626 126,

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CERTIFICATE

This is to certify that this dissertation entitled "**Antithyroid And Antioxidant Effects Of *Boerhaavia Diffusa* Linn on the L-Thyroxine Induced Hyperthyroidism In Rats"** by **Reg No: 261325351** was carried out in the pharmacology Arulmigu Kalasalingam College of Pharmacy, under the supervision and guidance of **Mr.N.R.Livingston raja, Assistant Professor** A.K.College of Pharmacy Anand Nagar, Krishnankoil.

I recommend this piece of work for acceptance as project for the partial fulfillment of the degree of **"MASTER OF PHARMACY"** of the Tamil Nadu Dr.M.G.R.Medical University, Chennai, for the academic year 2014 – 2015.

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Date :

Signature of Project guide
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EVALUATION SHEET

This dissertation work entitled **"Antithyroid And Antioxidant Effects of *Boerhaavia Diffusa* Linn on the L-Thyroxine Induced Hyperthyroidism In Rats"** was evaluated for the parial fulfillment of the requirement for the degree of **"MASTER OF PHARMACY"** in the Tamil Nadu Dr.M.G.R.Medical University.

Centre for evaluation: ----------

Examiners:

1.

2.

DEDICATED TO

GOD

AND

MY HUSBAND

Dr. D. Stephen,
Lecturer
Department of Botany

The American College,
Madurai-2

CERTIFICATE

This is to certify that the plant specimen brought to me by **Mrs.K. SELVA** II year **M. Pharm (Pharmacology); Student of ARULMUGU KALASALINGAM COLLEGE OF PHARMACY,** KRISHNANKOVIL has been identified as ***Boerhaavia diffusa linn*** Belonging to the family **Nyctaginaceae.**

Dr.D.Stephen.

Date ; 06/08/2014

Madurai

Tamil nadu

THE AMERICAN COLLEGE MADURAI DEPARTMENT OF BOTANY

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K.Selva

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1.1.THYROID GLAND

The thyroid gland is one of the largest endocrine glands. The thyroid gland produces two related hormones, **Thyroxine**(T_4) and **Triiodothronine** (T_3). Acting through nuclear receptors, these hormones play a critical role in cell differentiation during development and help maintain thermogenic and metabolic homeostasis in the adult.

The thyroid gland is a butterfly-shaped organ and is composed of two cone-like lobes or wings, lobus dexter (right lobe) and lobus sinister (left lobe). The two lobes are connected by an isthumus located inferior to the cricoid cartilage.

The organ is situated on the anterior side of the neck, lying against and around the larynx and trachea, reaching posterior to the oesophagus and carotid sheath. It starts cranially at the oblique line on the thyroid cartilage (just below the laryngeal prominence, or 'Adam's Apple'), and extends inferiorly to approximately the fifth or sixth tracheal ring. It is a highly vascular gland that weighs about 12 to 20g and is surrounded by a fibrous capsule. The lobes are roughly cone-shaped, about 5 cm long and 3 cm wide. Four parathyroid glands producing parathyroid hormone are located in the posterior region of each lobe of thyroid.[1, 2]

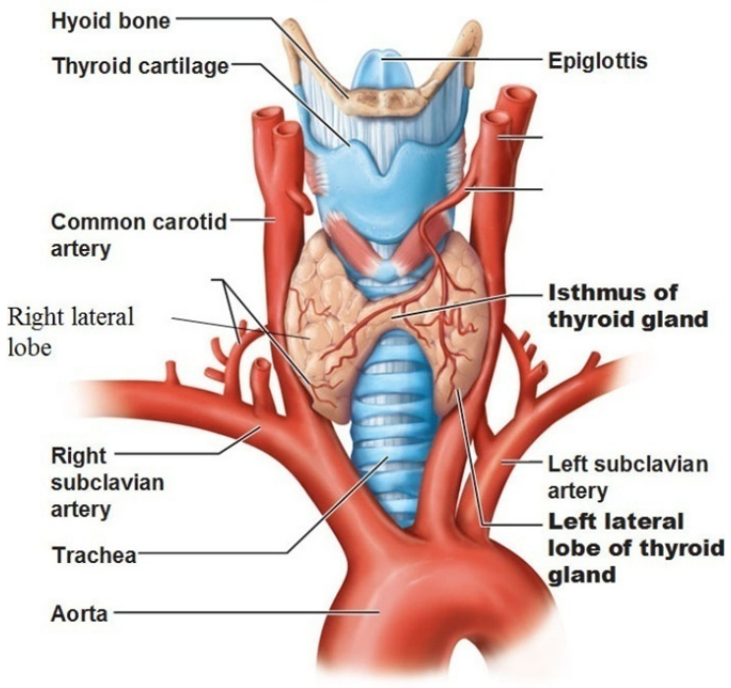


Fig NO 1: GROSS ANATOMY OF THE THYROID GLAND

The arterial blood supply to the gland is through the superior and inferior thyroid arteries. The superior thyroid artery is a branch of the external carotid artery and the inferior thyroid artery is a branch of the subclavian artery. The venous return is by the thyroid veins which drain into the internal jugular veins.

The thyroid hormone synthesis normally begins at about 11 week's gestation. The thyroid gland development is controlled by a series of developmental transcription factors like thyroid transcription factor 1(TTF1), thyroid transcription factor 1(TTF2) and paired homeobox-8(PAX-8). In combination, they orchestrate thyroid cell development and induction of thyroid-specific genes such as thyroglobulin (Tg), thyroid peroxidise (TPO), the sodium iodide symporter (NIS) and the thyroid-stimulating hormone receptor (TSH-R).

The thyroid gland is composed of large numbers of closed follicles (100 to 300 micrometers in diameter) filled with a secretory substance called colloid and lined with cuboidal epithelial cells that secrete into the interior of the follicles. The major constituent of colloid is the large glycoprotein thyroglobulin, which contains the thyroid hormones within its molecule.

Once the secretion has entered the follicles, it must be absorbed back through the follicular epithelium into the blood before it can function in the body. The thyroid follicular cells are polarized –the basolateral surface is apposed to the blood stream and an apical surface faces the follicular lumen. . Between the follicles there are other cells found singly or in small groups: parafollicular cells, also called C-cells, which secrete the hormone calcitonin an important hormone for calcium metabolism.[3-5]

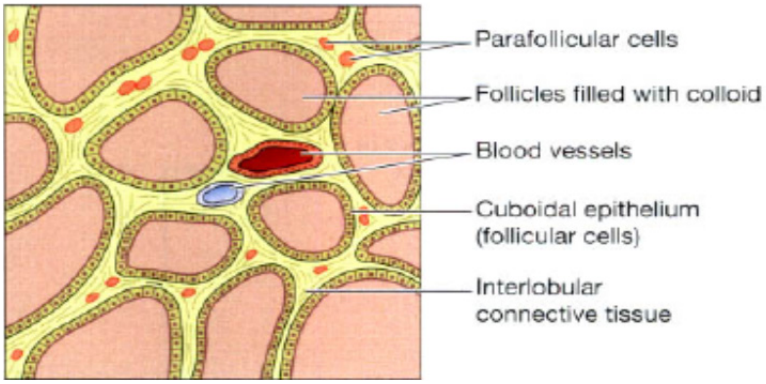


FIG NO: 2 MICROSCOPIC STRUCTURE OF THE THYROID GLAND

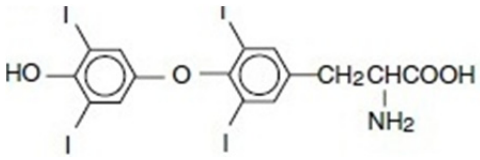
1.2.THYROID HORMONE

The thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) are tyrosine based hormones produced by thyroid gland that are primarily responsible for the regulation of metabolism.

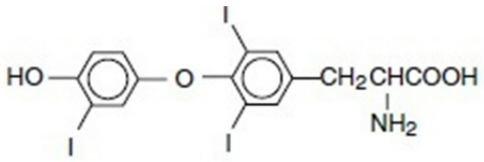
The thyroid hormones T_3 and T_4 are formed in a large prohormone molecule-thyroglobulin, the major component of the thyroid gland. Thyroglobulin is synthesized by the endoplasmic reticulum and golgi apparatus in the thyroid follicular cells and secreted into the lumen of the follicles. It is a large glycoprotein (660,000 daltons) made up of two identical subunits, each with a molecular weight of 330,000 daltons. It is of special importance because it is necessary for the synthesis of thyroid hormones and represents their form of storage.

Each molecule of thyroglobulin contains about 70 tyrosine amino acids, and they are the major substrates that combine with iodine to form the thyroid hormones. The thyroxine and triiodothyronine hormones formed from the tyrosine amino acids remains as a part of the thyroglobulin molecule during synthesis of the thyroid hormones and even afterward as stored hormones in the follicular colloid.

About 93 per cent of the metabolically active hormone secreted by the thyroid gland is thyroxine and 7 percent triiodothyronine. [6, 7]



THYROXINE



3, 5, 3′-TRIIODOTHYRONINE

THYROID HORMONE STRUCTURE

1.3. REGULATION OF THYROID HORMONE PRODUCTION

The production of thyroid hormones is regulated by the hypothalamic-pituitary thyroid axis. Thyrotropin-releasing hormone (TRH) is synthesized in the hypothalamus and stimulates the release of thyroid-stimulating hormone (TSH) from the anterior pituitary. Secretion of TRH is stimulated by exercise, stress, malnutrition, low plasma glucose and sleep.

TSH is regulated by the negative feedback of the thyroid hormones; thyroxine (T_4) and triiodothyronine (T_3) acting on the pituitary gland and probably the hypothalamus i.e. increased levels of T_3 and T_4 decrease TSH secretion and vice versa. TSH binds to TSH-receptor (TSH-R) on the thyroid cell membrane, a GPCR which stimulates the G_s-adenylyl cyclase-cyclic AMP pathway. Thus stimulation of the receptor results in increased cAMP formation which mediates increase in uptake and transport of iodide, iodination of thyroglobulin, and synthesis of iodotyrosines. TSH binding to TSH-R also stimulates phospholipase C leading to thyroid cell hypertrophy. Chronic TSH stimulation causes entire gland to hypertrophy causing a goiter in the case of iodine deficiency.[8-10]

1.4. BIO SYNTHESIS OF THYROID HORMONES

The formation of the thyroid hormones depends on an exogenous supply of iodide. The thyroid gland is unique in that it is the only tissue of the body able to accumulate iodine in large quantities and incorporate it into hormones.

Iodine-Essential for Thyroid Hormone Synthesis

- Iodine is an essential raw material for thyroid hormone synthesis.
- The minimum daily iodine intake that will maintain normal thyroid function is 150µg.
- Average dietary intake is approximately 500µg/day.
- About 120µg/day enter the thyroid.
- The thyroid secretes 80µ/day in the form of T3 and T4.
- 40µg/day diffuses back into the extracellular fluid (ECF).

The major steps in the synthesis, storage, release and inter-conversion of thyroid hormones are:

- **ACTIVE UPTAKE OF IODIDE BY THE FOLLICULAR CELLS**

- The first stage in the formation of thyroid hormones is transport of iodides from the blood into the thyroid glandular cells and follicles.
- The basal membrane of the thyroid cell has the specific ability to pump the iodide actively to the interior of the cell. This is called **iodide trapping**.
- The Basolateral membrane of thyroid cell possesses a pump called $Na+/I^-$ symporter(NIS), which transports two Na+ ions and one I^- ion into the cell with each cycle, against the electrochemical gradient for I^-.[11-13]

- **OXIDATION AND IODINATION**

- The oxidation of iodide to its active form of iodine and the iodination of tyrosine are catalyzed by thyroid peroxidase, a heme-containing glycoprotein bound to the apical membrane of thyroid cells that utilizes hydrogen peroxide (H_2O_2) as the oxidant.
- This iodine is then capable of combining directly with the amino acid tyrosine residues on the thyroglobulin (**organification**)
- This process occurs at the apical membrane of thyrocytes, facing the colloid.
- Tyrosine is first iodized to monoiodotyrosine and then to diiodotyrosine.[14-20]

- **COUPLING REACTION**

- The final step in the process of hormone synthesis is the coupling of two appropriate iodotyrosine residues, giving an iodothyronine residue
- T_4 is formed by condensation of two molecules of DIT.
- T_3 is formed by condensation of MIT with DIT.
- The reaction is catalyzed by thyroid peroxidase (TPO) and requires H_2O_2 which is the co-factor for TPO.[21,22]

➢ STORAGE OF THYROID HORMONES

- The thyroid gland is unusual among the endocrine glands in its ability to store large amounts of hormone.
- Each thyroglobulin molecule contains up to 30 thyroxine molecules and a few triiodothyronine molecules.
- Stored Thyroid Hormones maintain the body's requirement of T3 and T4 for up to 2-3 months.

➢ RELEASE OF THYROID HORMONES

- Thyroglobulin is secreted into the circulation by Proteolysis.
- Thyroglobulin appears as intracellular colloid droplets (endocytosis) which apparently fuse with lysosomes containing the requisite proteolytic enzymes.
- Protease enzymes digest the thyroglobulin molecules and release T_3 and T_4 and diffuse through the base of the thyroid cell into the blood.
- About 93 per cent of the thyroid hormone released from the thyroid gland is normally thyroxine; only 7 per cent is triiodothyronine.

➢ PERIPHERAL CONVERSION OF T_4 TO T_3

- Mono-deiodination of T_4 in peripheral tissues accounts for about 80% of circulating T_3.
- The major nonthyroidal site of conversion of T_4 to T_3 is the liver
- Removal of the 5′-or outer ring iodine leads to the formation of T_3 in the "activating" metabolic pathway.
- The type I 5′-deiodinase (D1) is expressed in the liver, kidney and thyroid; generates circulating T_3 that is used by most peripheral target tissues.
- Type II 5′-deiodinase (D2) is expressed in the brain, pituitary, skeletal and cardiac muscle; supplies intracellular T_3 to these tissues.[23,24]

➢ TRANSPORT

- The released hormones in the circulation bind to albumin, thyroid-binding prealbumin (TBPA) and thyroid-binding globulin (TBG).
- The TBG has a high affinity for T_3 and lower affinity for T_3.
- The unbound T_4 and T_3 are the biologically active forms of the hormones.
- Thyroid hormones are taken into cells by facilitated diffusion or by active transport secondary to a sodium gradient.
- A great part of T_4 is stored in liver and kidney, whereas most T_3 appears in muscle and brain.

➢ DEGRADATION AND EXCRETION

- Degradative metabolism of the thyroid hormones occurs mainly in the liver, where both T_3 and T_4 are conjugated to form either glucuronide (mainly T_4) or sulfate (mainly T_3) through the phenolic hydroxyl group.
- The resulting iodothyronine conjugates are excreted via the bile into the intestine.
- It also undergoes marginal enterohepatic circulation and is excreted unconjugated in faeces.[25]

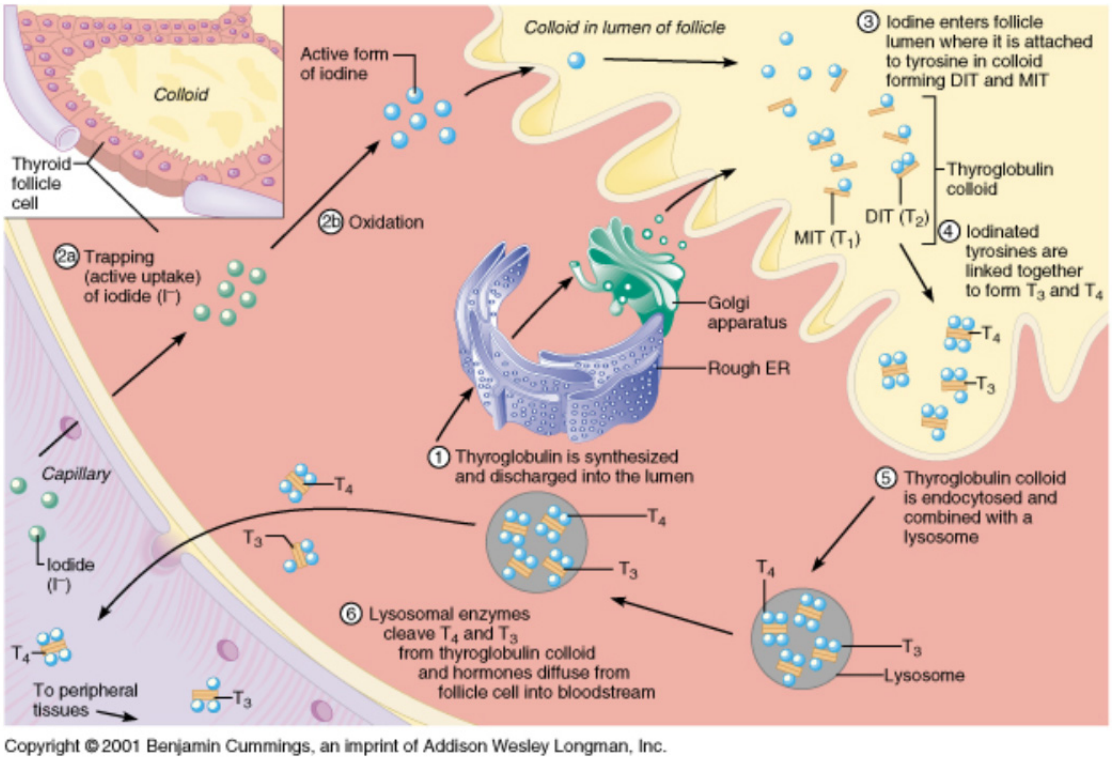
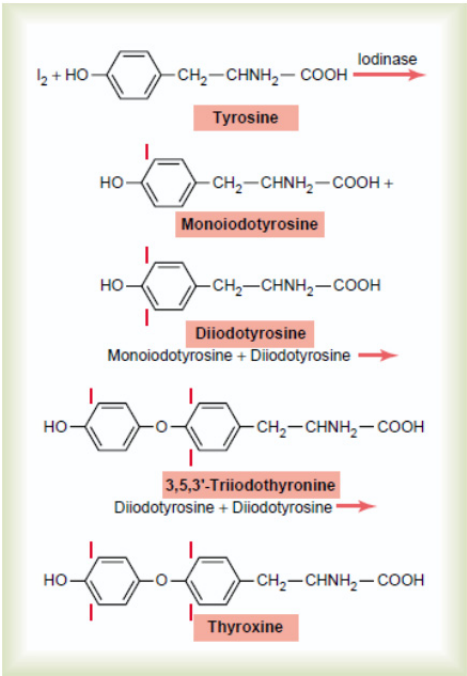


FIG NO 3: BIOSYNTHESIS OF THYROID HORMONE



CHEMISTRY OF T_3 AND T_4

1.5. THYROID HORMONE RECEPTOR

The thyroid hormone receptor (TR) is a type of nuclear receptor. Thyroid hormones act by binding to nuclear thyroid hormone receptors (TRs) α and β. Both the receptors are variably spliced to form unique isoforms. There are three isoforms of the thyroid hormone receptor designated as α-1, β-1 and β-2 that are able to bind thyroid hormone. The relative levels of expression of the isoforms among the organs are

- TR-α1 –expressed in cardiac and skeletal muscles
- TR-β1 -expressed in brain, liver and kidney
- TR-β2 -expression primarily limited to the hypothalamus and pituitary

The thyroid receptor contains a central DNA-binding domain and a C-terminal ligand binding domain. They bind to specific DNA sequences named Thyroid Response Elements (TREs), in the promoter region of the target genes. The receptor binds as homodimer or as heterodimers with retinoic acid X receptors (RXRs). The activated receptor can either stimulate gene transcription or inhibit transcription depending on the nature of regulatory elements in the target genes.

Binding of thyroid hormone, results in a conformational change in TR which displaces co-repressor from the receptor/DNA complex and allows the recruitment of co-activator proteins. The DNA/TR/co-activator complex thus enhances transcription by recruiting RNA polymerase that transcribes downstream DNA into messenger RNA and eventually new proteins are synthesized, that results in a change in cell function. . In the absence of hormone, TR binds with co-repressor proteins that inhibit gene transcription.[26, 27]

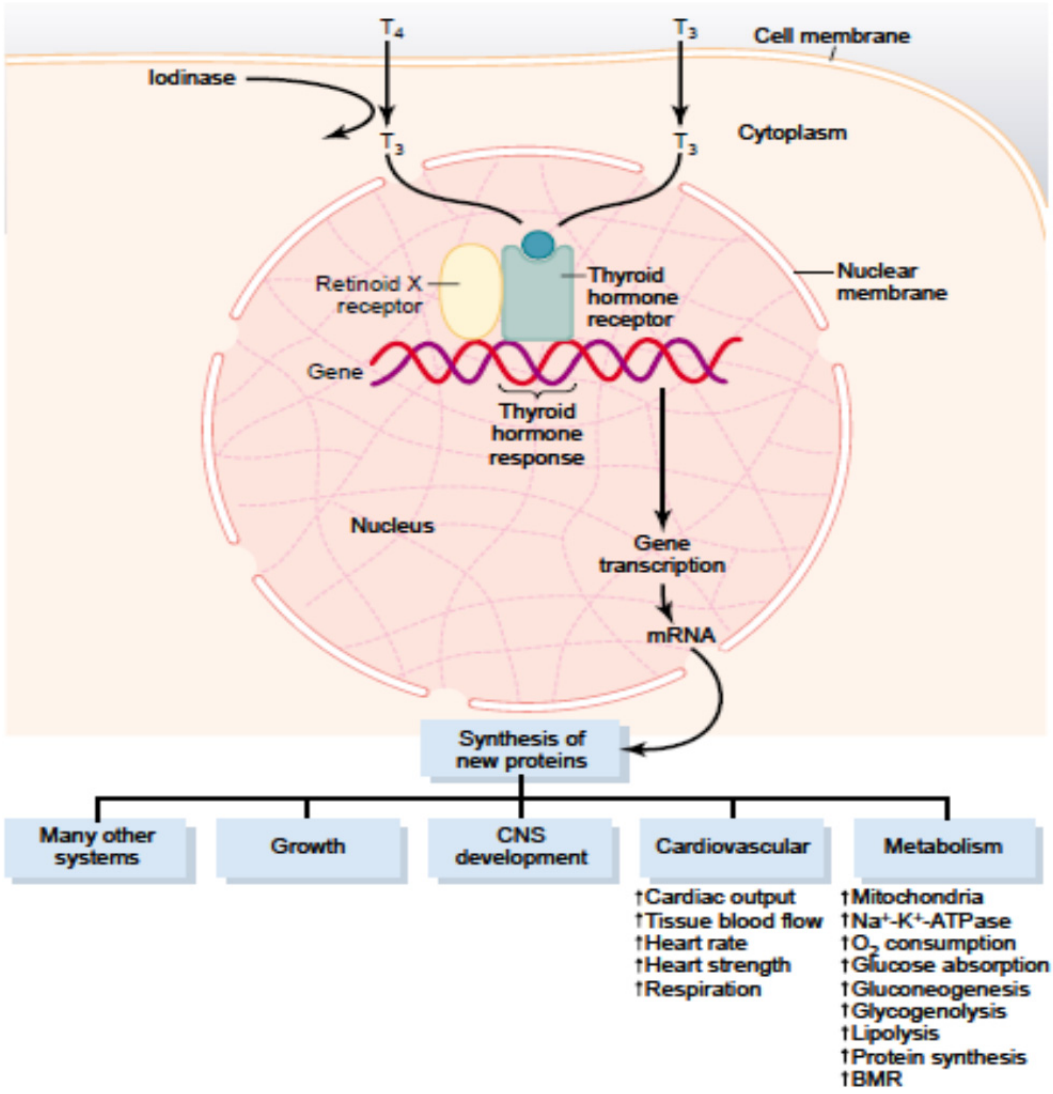


FIG NO 4: THYROID HORMONE RECEPTOR:

T_4 and T_3 readily diffuse through the cell membrane. Much of the T_4 is deiodinated to form T_3, which interacts with the thyroid hormone receptor, bound as a heterodimer with a retinoid X receptor, of the thyroid hormone response element of the gene. This causes either increases or decreases in transcription of genes that lead to formation of proteins, thus producing the thyroid hormone response of the cell. The actions of thyroid hormone on cells of several different systems are shown.

1.6. PHYSIOLOGIC FUNCTIONS OF THE THYROID HORMONES

The general effect of thyroid hormone is to activate nuclear transcription of large numbers of genes. Therefore, in virtually all cells of the body, great numbers of protein enzymes, structural proteins, transport proteins, and other substances are synthesized. The net result is generalized increase in functional activity throughout the body.[28]

Effect on Carbohydrate Metabolism

- Stimulates almost all aspects of carbohydrate metabolism
- Stimulates uptake of glucose by the cells
- Stimulates glycolysis and gluconeogenesis
- Stimulates rate of absorption from GIT

Effect on Fat Metabolism

- Fat metabolism is enhanced under the influence of thyroid hormone
- Decreases concentration of cholesterol, phospholipids, and triglycerides in plasma
- Increases free fatty acid concentration in the plasma and accelerate the oxidation of free fatty acids by cells.[29]

Effect on Basal Metabolic Rate

- Increases BMR
- The basal metabolic rate (BMR) can increase to 60 to 100 per cent above normal
- The rate of protein synthesis is increased, at the same time rate of protein catabolism is also increased

Effect on Body Weight

- Decreases the body weight

Effect on Growth

- Promotes growth and development of brain during foetal life
- Induces growth of bones

Effect on the Cardiovascular System

- Normalize mean arterial blood pressure
- Increases blood flow and cardiac Output
- Increases heart rate[30]

Effect on Respiration

- Increase rate and depth of respiration

Effect on GIT

- Stimulates secretion of digestive juices
- Stimulates motility of the gastrointestinal tract
- Stimulates appetite[31]

Effect on Muscles

- Stimulates muscles and react with vigor
- When the quantity of hormone becomes excessive, muscles become weakened because of excess protein catabolism

Effect on Sexual Function

- For normal sexual function, thyroid secretion needs to be approximately normal.
- In men, lack of thyroid hormone is likely to cause loss of libido; great excesses of the hormone, sometimes cause impotence
- In women, lack of thyroid hormone often causes menorrhagia and polymenorrhea and excess of hormone causes oligomenorrhea.[32]

Effect on Other Endocrine Gland

- Increases secretion of most other endocrine glands
- Increases secretion of insulin due to excessive glucose metabolism
- Increases secretion of parathyroid hormone
- Increases glucocorticoid secretion from adrenal gland[33]

1.7. DISORDERS OF THE THYROID

Thyroid diseases are very common types of endocrine disorder, particularly in middle aged and elderly women.

The types of disorders which are associated with the abnormal secretion of thyroid hormones are

- **Hyperthyroidism**: a condition where there is an increase in production of thyroid hormones due to excessive thyroid function.[34]
- **Hypothyroidism**: a condition where there is a decrease in production of thyroid hormones due to impaired thyroid function. [35]

Hormones	Normal Range	Interpretation
TT_4	64-142mmol/L	>142mmol/L indicates hyperthyroidism and <64 mmol/L indicates hypothyroidism
TT_3	1.46-2.92mmol/L	>2.92mmol/L indicates hyperthyroidism and <1.46 indicates hypothyroidism
FT_4	12-26pmol/L	>26pmol/L indicates hyperthyroidism and < 12 pmol/L indicates hypothyroidism
FT_3	0.22-6.78pmol/L	>6.78 pmol/L indicates hyperthyroidism and < 0.22 pmol/L indicates hypothyroidism
TSH	0.4-4.8mIU/L	<0.4 mIU/L indicates hyperthyroidism and >0.4 mIU/L indiactes hypothyroidism

Table No: 1 DISORDERS OF THE THYROID

1.8. HYPERTHYROIDISM

Hyperthyroidism, often referred to as an 'overactive thyroid', is a condition in which the thyroid gland produces and secretes excessive amounts of the free thyroid hormones: triiodothyronine (T_3) and/or thyroxine (T_4).

Thyrotoxicosis is defined as the state of elevated serum levels of T_4 (>142mmol/L) and/or T_3 (>2.92mmol/L) which is caused due to hyperthyroidism.[36]

1.9. EPIDEMIOLOGY

The most common cause of hyperthyroidism in the western world is Graves' disease, which accounts for 90% of cases. This form of hyperthyroidism may affect any age group, but it is uncommon in childhood and most frequent in the third to fifth decades. Women are affected about 10 times more than men. In the UK the prevalence of overt hyperthyroidism is 20 per 1000 females and 2 per 1000 males.[37]

According to British medical bulletin, the incidence data available for overt hyperthyroidism in men and women from large population studies are comparable, at 0.4 per 1000 women and 0.1 per 1000 men, but the age-specific incidence varies considerably. The peak age-specific incidence of Graves' disease was between 20 and 49 years in two studies, but increased with age in Iceland and peaked at 60–69 years in Sweden. The peak age-specific incidence of hyperthyroidism caused by toxic nodular goiter and autonomously functioning thyroid adenomas in the Sweden study was >80 years. The only available data in a black population, from Johannesburg, South Africa, also suggest a 10-fold lower annual incidence of hyperthyroidism (0.09 per 1000 women and 0.007 per 1000 men) than in whites.[38]

The incidence of hyperthyroidism from toxic multi nodular goiters (TMG) ranges from 9% to 16% per 100,000 has been reported. This is the most common cause of new-onset hyperthyroidism in adults in the fifth or sixth decade of life. Single toxic adenomas are less common; an incidence of 12.6% was noted in the United States, and a higher incidence of 9% was noted in Europe. Post partum thyroiditis develops in 4% to 8% of women after delivery and in as many as 25% of women with insulin-dependent diabetes.

The prevalence of hyperthyroidism has been studied in several studies. In an epidemiological study from Cochin, subclinical and overt hyperthyroidism were present in 1.6% and 1.3% of subjects participating in a community survey. In a hospital-based study of women from Pondicherry, subclinical and overt hyperthyroidisms were present in 0.6% and 1.2% of subjects. More than a third of community-detected hyperthyroid cases have positive anti-TPO antibodies, and about 39% of these subjects have a goiter.

Population studies have suggested that about 16.7% of adult subjects have anti-thyroid peroxidase (TPO) antibodies and about 12.1% have anti-thyroglobulin (TG) antibodies. In this same study of 971 subjects, when subjects with abnormal thyroid function were excluded, the prevalence of anti-TPO and anti-TG antibodies was 9.5% and 8.5%.

The Indian Council of Medical Research established the National Cancer Registry Program, and the NCRP has collected the data of more than 3, 00,000 cancer patients between the periods 1984 and 1993. Among these patients, the NCRP noted 5614 cases of thyroid cancer, and this included 3617 females and 2007 males. The six centers involved in the studies were at Mumbai, Delhi, Thiruvananthapuram, Dibrugarh, Chandigarh, and Chennai. Among them, Thiruvananthapuram had the highest relative frequency of cases of thyroid cancer among all cancer cases enrolled in the hospital registry, 1.99% among males and 5.71% among females. The nationwide relative frequency of thyroid cancer among all the cancer cases was 0.1%–0.2%. The age-adjusted incidence rates of thyroid cancer per 100,000 are about 1 for males and 1.8 for females as per the Mumbai Cancer Registry, which covered a population of 9.81 million subjects. [39]

1.10. AETIOLOGY

The most common cause of this syndrome is Grave's disease, followed by toxic multinodular goitre and hyperfunctioning solitary nodules. Autoimmune postpartum and subacute thyroiditis, tumors that secrete thyrotropin and drug–induced thyroid dysfunction are other important causes.[40]

1. **Grave's disease:**

This is the most common cause of hyperthyroidism and is an autoimmune disorder. In this disease, the patient produces auto-reactive IgG antibodies that have an antigen binding-site, which is complementary to the TSH-receptor found on the cell surface membrane of thyroid follicle cells. These IgG antibodies are called ***thyroid stimulating immunoglobulin*** (TSI) and bind to TSH- receptor. The TSI are synthesized in the thyroid gland, bone marrow and lymph nodes. These antibodies hyper-stimulates the receptor and consequently triggers follicle cell hypertrophy (toxic goitre), and causes excessive TH synthesis/secretion (thyotoxicosis).[41]

A combination of genetic factors including HLA-DR and CTLA-4 polymorphisms and environmental factors contribute to the development of TSI.[42]

2. **Toxic multinodular goiter:** (TMG)

TMG is a condition that affects elderly females who previously had normal thyroid function. It is the second most common cause of hyperthyroidism after Grave's disease. This type of hyperthyroidism is common in areas of the world with iodine deficiency In TMG the thyroid gland contains many overactive nodules that secrete excessive TH, independently to the influence of TSH. [43]

TMG usually starts as a non-toxic multinodular goitre, which is formed via the hyperplastic response of the thyroid gland to iodine deficiency. Over the time, the non-toxic goitre becomes functional and begins to secrete TH hormone.[44]

It is not clearly known why the non-toxic multinodular goitre becomes TMG and one theory suggests that the somatic mutations of TSH- receptors found on the cells of the nodules causes the autonomous secretion of TH.[45]

3. HYPERFUNCTIONING SOLITARY NODULES:

A solitary, autonomously hyperfunctioning thyroid nodule is referred to as **toxic adenoma**. It's also called as plummers disease. Thyroid adenoma is a benign tumour of the thyroid gland, which has originated via somatic mutations of the thyrotropin, or thyroid-stimulating hormone (TSH) receptor.The nodule is usually 3 to 5cm diameter and is not under TSH regulation. This nodule can produce normal or supra-physiologic amounts of thyroid hormone causing suppression of existing normal thyroid tissue.[46]

In over 85% of cases, the tumour is silent and the patient is euthyroid. However, in about 10% of patients, the adenoma is functional and secretes excessive TH which results in symptomatic hyperthyroidism. When functional, the condition is known as toxic thyroid adenoma.[47]

4. THYROIDITIS:

Thyroiditis refers to the inflammatory condition of the thyroid gland. Results of several investigations have shown that thyroiditis, whether autoimmune, infectious or toxic affects apoptotic pathways leading to thyroid follicular cell death. This apoptosis leads to follicular disruption and release of hormonal stores into the circulation, resulting in hyperthyroidism. After the inflammatory process subsides, mild to moderate hypothyroidism generally follows, before complete recovery.[48]

Thyroiditis does not cause the thyroid to produce excess hormone. Instead, it causes stored thyroid hormone to leak out of the inflamed gland and raise hormone levels in the blood.[49]

Thyroiditis is classified into

a) **Acute Thyroiditis:**

Acute thyroiditis is very rare and is caused mainly due to bacterial and fungal infections of the thyroid.

b) **Subacute Thyroiditis: (**Quervain's Thyroiditis/Granulomatous Thyroiditis/Viral Thyroiditis)

This condition involves painful inflammation and enlargement of the thyroid gland. This is caused mainly due to viral infections. The condition usually goes away on its own in a few months. Many people with subacute thyroiditis briefly develop hypothyroidism before the thyroid gland is completely healed.

c) **Silent Thyroiditis:**

This type of thyroiditis is called "silent" because it is painless and occurs in patients with underlying autoimmune thyroid disease. It's also called post partum thyroiditis since condition occurs in up to 5% of women 3 to 6 months after pregnancy. Hyperthyroidism in this condition usually lasts for approximately 1 to 2 months. As with subacute thyroiditis, women with postpartum thyroiditis often develop hypothyroidism before the thyroid gland is completely healed. In some women, the gland does not heal and hormone levels remain low. These women must take thyroid hormone replacement for the rest of their lives.

5. Drug-Induced Hyperthyroidism:

Drug induced hyperthyroidism is mainly caused in abnormal thyroid gland that have lost the protective Wolff-Chaikoff block. There are 2 main drugs that cause hyperthyroidism in normal patients:

- **Amiodarone**: This is an antiarrhythmic drug. The structure of amiodarone is similar to TH, and it can therefore bind to the TH-receptors that are found intra-cellularly in many organs, thus inducing hyperthyroid symptoms.

- **Exogenous iodine** ((Jod-Basedow Phenomenon): Iodine-induced thyrotoxicosis is seen commonly in residents of iodine-deficient areas who became hyperthyroid after taking excessive iodine supplements, or by eating lots of iodine-rich food.

6. Thyrotoxicosis Factitia:

This is a very uncommon type of hyperthyroidism that is caused by the excessive ingestion of exogenous TH. This may be due to an accidental thyroxine overdose in patients with hypothyroidism.

7. hCG-Induced Hyperthyroidism

Human chorionic gonadotrophin (hCG) is a hormone that is usually secreted by an embryo in pregnancy. In pregnancy, its main function is the maintenance of the corpus luteum in the ovary, thereby maintaining high progesterone levels that are necessary for a normal pregnancy.

The structure of hCG is very similar to that of TSH and hCG binds to the TSH receptors on the thyroid follicle cells; stimulating the secretion of TH. Hence, excessive hCG levels cause hyper-secretion of TH, and subsequent hyperthyroidism.

There are two main conditions that raise plasma hCG concentrations and thus cause hyperthyroidism:

- **Pregnancy**: hCG is raised physiologically in pregnancy, and this can cause a transient, pregnancy-induced hyperthyroidism.
- **Tumours**: Some tumours secrete large quantities of hCG, and this can cause hyperthyroidism.

8. Thyroid Cancer

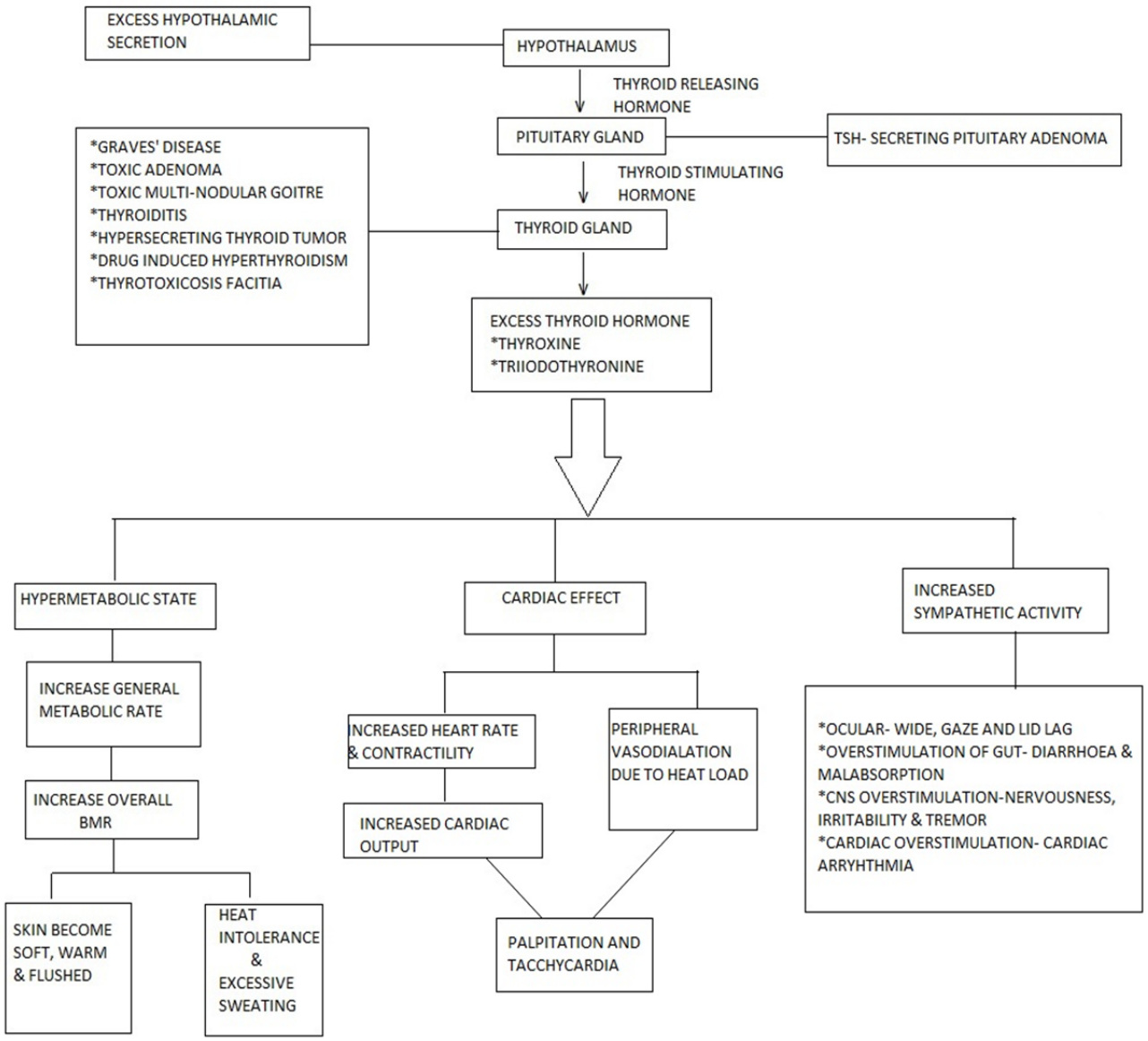
Thyroid carcinoma is the most common malignancy of the endocrine system. Most malignant tumours of the thyroid gland secrete excessive TH and can thus cause hyperthyroidism. There are two types of thyroid gland carcinoma that cause excess TH secretion:

- **Follicular thyroid cancer (FTC):** This is a primary tumour of the thyroid gland, which originates from the follicle cells.
- **Metastatic differentiated thyroid carcinoma:** This is a highly differentiated thyroid gland malignancy that has metastasised from another site in the body (the main primary tumours that metastasise to the thyroid are from the kidneys, lungs, breast, oesophagus or uterus).

CAUSES	PATHOPHYSIOLOGY
PRIMARY HYPERTHYROIDISM	
• Graves Disease	Increased glandular stimulation
• TMN Goitre	Autonomous hormone production
• Toxic adenoma	Autonomous hormone production
• Struma Ovarii	Extra glandular production
• Jod-Basedow	Increased glandular stimulation
• Sub-Acute Thyroiditis	Leakage of hormone from the gland
• Silent Thyroiditis	Leakage of hormone from the gland
• Thyrotoxicosis Factitia	Exogenous hormone intake
SECONDARY HYPERTHYROIDISM	
• hCG Induced Thyrotoxicosis	Increased glandular secretion

TABLE NO: 1.1

1.11. PATHOPHYSIOLOGY



PATHOPHYSILOGY[50]

1.12. SIGNS AND SYMPTOMS

The clinical manifestations of hyperthyroidism are directly related to increased TH secretion. The majority of the signs and symptoms are just exaggerated forms of the normal effect of TH, and these include: [51,52]

TABLE NO:2

Skin and Appendages	Warm and moist skin, Thinning or loss of hair, Exophthalmos (protrusion of the eyeball), Lid retraction, Lid lag, Increased sweating, Heat intolerance, Ophthalmoplegia, Plummers nail, Palmar erthyma, Peritibial myxodema
Nervous system	Insomnia, Irritability, Nervousness, Anxiety, Psychosis (rarely), Choreoathetosis, Hyperkinesis (children)
Musculoskeletal	Osteoporosis, Muscle weakness, Tremor, Rapid Deep tendon reflexes, Fatigue
Gastro intestinal	Weight loss with increased appetite, Diarrhoea
Cardio vascular	Palpitations, Tachycardia, Angina , Atrial fibrillation
Genito urinary	Amenorrhoea
Neck	Goitre

1.13. DIAGNOSIS OF HYPER THYROIDISM

Several laboratory tests are available to assess thyroid homeostasis and metabolic function. These tests evaluate circulating hormone levels, glandular activity, hypothalamic – pituitary function, autoimmunity and various non-specific metabolic indices.

➢ Circulating Hormone Levels

Total T_3:

- Total T_3 tests are often useful to diagnosis hyperthyroidism or to determine the severity of the hyperthyroidism.
- Serum concentration of total T_3 is measured by radioimmunoassay method.
- Hyper thyroid patients have T_3 >2.92 mmol/L

Total T_4:

- Serum total T4 concentration is measured by radioimmunoassay.
- This test measures the total t4 in blood i.e.; both the bound and free form of the hormone.
- Hyper thyroid patients have T_4 > 142mmol/L

Several laboratories measure the total T4 and total T3 which is not a true reflection of the thyroid status of an individual. This is because thyroid hormones circulate in the body largely in the inactive form, bound to carrier proteins (thyroid binding globulin (TBG), transthyretin and albumin) while only the small unbound fraction is metabolically active. The development of newer immunoassay methods for determining free T3 and T4 has overcome many of these problems. [53]

Free Thyroxin (FT_4):

- The free thyroxin can be measured by equilibrium dialysis or by analog type radio immune assays.
- It's a direct method of measurement.
- By employing this technique, TBG's variable effect on total T4 concentration is eliminated, thereby affording more precise estimates of thyroid status.
- Hyper thyroid patients have FT4 >12-26pmol/L.[54]

Free Triiodothyronine (FT_3):

- The free thyroxin can be measured by immunoenzymatic assay.
- It's a direct method of measurement.
- The concentration of FT_3 increases in the case of hyper thyroidism.
- Hyper thyroid patients have FT3 > 0.22-6.78 pmol/L

- **Glandular Activity**

Radio Active Iodine Uptake (RAIU) and Thyroid Scanning:

RAIU measures only the iodine trapping ability of the gland without regard to the iodine's ultimate rate. After the tracer dose of radioactive iodine, the percentage of iodine uptake is measured at 5 and at 24 hours. An elevated update of >35% at 24 hours occurs in hyperthyroidism. The most commonly used isotopes of iodine are iodine 123 I,125 I,131 I and the thyroid gland uptake these isotopes. After the uptake nuclear imaging of the patient is done.

- Grave's disease is characterized by an enlarged gland and increased tracer uptake that is distributed homogeneously.
- Toxic adenomas appear as focal areas of increased uptake, with suppressed tracer uptake in the remainder of the gland.
- Sub acute thyroiditis is associated with very low update because of follicular damage and TSH suppression
- In toxic multi nodular Goiter, there are multiple areas of relatively increased or decreased trace update.

A thyroid scan is usually done after RAIU or 99mTc pertechnetate uptake . This detects hypo functioning, non-iodine –concentrating, hyper functioning, hyper-iodine – concentrating areas in parts of or the whole gland . The thyroid scan shows how the iodine is distributed in the gland.[(55)]

- **Hypothalamic – Pituitary Function**

The integrity of the negative feed-back hypothalamic pituitary axis is evaluated by the serum thyrotropin (TSH) and the thyrotropin – releasing hormone test (TRH). The TRH is mainly used for diagonosing hypothyroidism.

TSH or Thyrotropin Test:

A low TSH level in the blood is the most accurate indicator of hyper thyroidism. The body shuts off production of this pituitary hormone when the thyroid gland even slightly overproduces thyroid hormone. If the TSH level is low, it is very important to check the thyroid hormone levels to confirm the diagnosis of the hyper thyroidism. Recently, a new immunoassay methodology has been applied to the measurement of TSH. Depending on the type of label attached to the TSH antibody, the assay is variously called the immunoradiometric assay, the immunofluorimetric assay, the immunochemiluminometric assay, or the immunoenzymometric assay.

Hyperthyroid patients have TSH valus < 0.4mIU/L[56]

- **Test of Auto Immunity: Antibodies**

Antithyroglobulin (ATgA) and Anti Microsomal Antibodies (AMA):

The two main antigens are identified in the thyroid gland are antithyroglobulin(ATgA) and anti-microsomal antibodies (AMA). The presence of these antibodies suggests an auto immune process such as Graves's disease and /or Hashimotos thyroiditis. The levels of ATgA are higher during the acute phase of auto immune thyroid disease and decline during remission and after therapy. The AMA is the more sensitive of the two antibodies because levels remain detectable after remission where as ATgA titres revert to normal.[57]

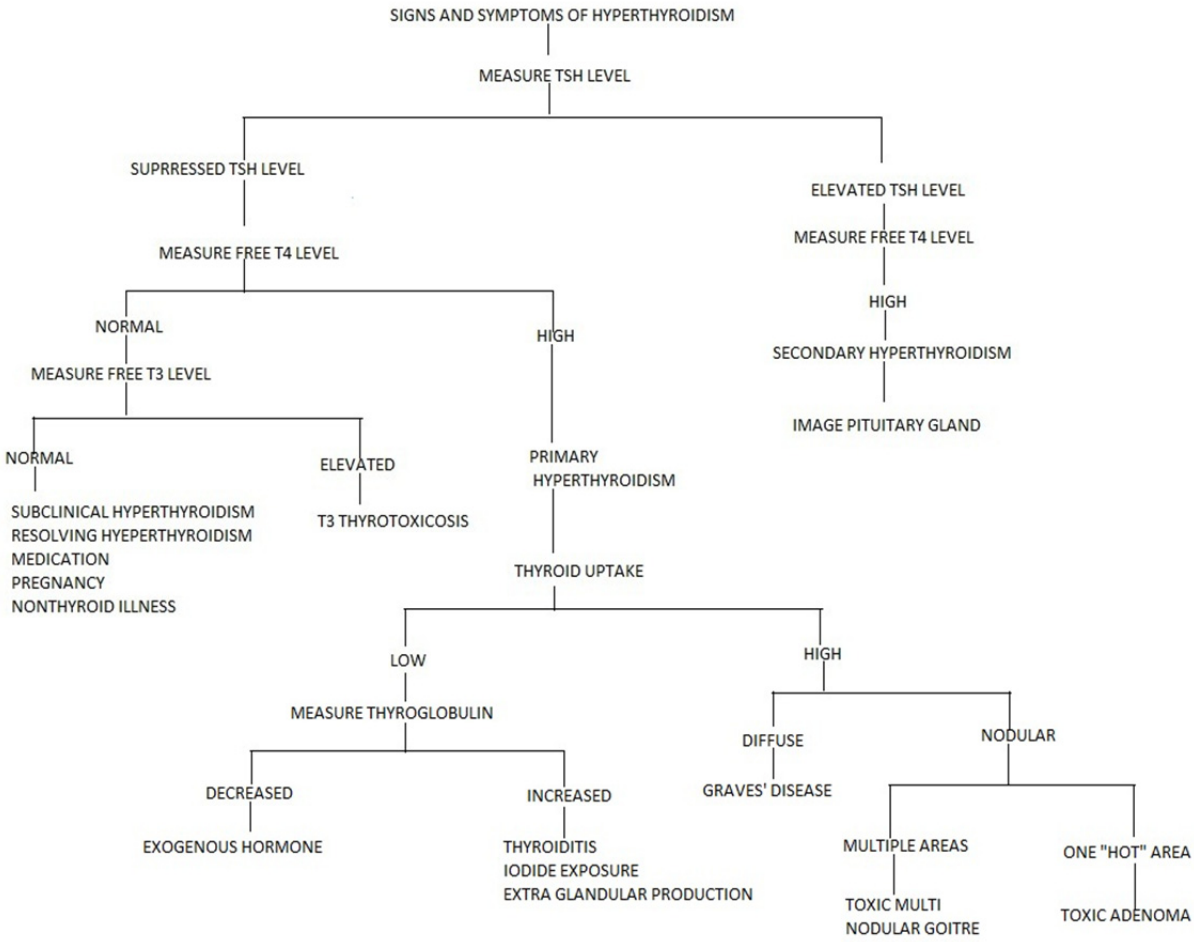
Thyroid Receptor Antibody (TRab)

An IgG immunoglobulin capable of stimulating the thyroid gland like TSH, is commonly present in 90% of patients with Grave's disease. The TRab will help to confirm the diagnosis of Grave's diseases in clinically euthyroid patients with an atypical presentation. A high maternal TRab level in pregnant female provides predictive information about the risk of neonatal Grave's disease.[58]

- **Non-Specific Indices**

Serum cholesterol, SGOT, Creatine, phosphokinase (CPK), and LDH levels are decresed in severe hyper thyroidism. [59]

1.14. DIAGONOSIS



1.15. TREATMENT

Hyperthyroidism may be treated pharmacologically or surgically. In general, surgery is used only when there are problems resulting from compression of the trachea, and it is usual to remove only part of the organ. Although the condition of hyperthyroidism can be controlled with antithyroid drugs, these drugs do not alter the underlying autoimmune mechanisms or improve the exophthalmos associated with Graves' disease.

A large number of compounds are capable of interfering, directly or indirectly, with the synthesis, release, or action of thyroid hormones. The major inhibitors are listed in table no:3

TABLE NO: 2.1

DRUGS USED FOR THE TREATMENT OF HYPERTHYROIDISM

1. Anti Thyroid drugs	Propyl-thiouracil, Methimazol, Carbimazole
2. Ionic Inhibitors	Potassium perchlorate, Thiocyanate
3. Iodine and Iodine-Containing Agents	Sodium Iodide, Potassium Iodide, Lugols Iodine, Oral Cholecystographic Agents
4. Radiation Therapy	Radioactive Iodine (Iodotope I-131)

1. **Anti Thyroid Drugs**:

They interfere directly with the synthesis of thyroid hormones. These drugs bind to and inhibit the thyroperoxidase; they thus block thyroid hormone synthesis by inhibiting:-

- Coupling of iodotyrosines
- Formation of MIT
- Conversion of MIT TO DIT

2. **Ionic Inhibitors:**

They act by blocking the iodide transport mechanism. These drugs are competitive inhibitor of thyroidal I^- transport via the Sodium Iodide Symporter (NIS). Thus they competitively inhibit trapping of iodide by the thyroid gland.

3. **Iodine And Iodine-Containing Agents:**

When administered in pharmacological amounts, iodides causes a transient inhibition of the uptake and incorporation of I^- into Tg . In addition, high doses of KI also inhibit the secretion of thyroid hormone and thyroid blood flow. These effects make KI an ideal agent for treating severe thyrotoxicosis or thyroid crisis when a rapid decrease in plasma T4 and T3 is desirable.

The iodine-containing oral cholecystographic contrast agents (OCAs) act by inhibiting the peripheral conversion of T_4 to T_3 and thus lower their concentration in the plasma and it includes

- Sodium ipodate (*Oragrafin*)
- Iopanoic Acid (*Telepaque*)
- Tyropanoic Acid (*Bilopaque*)
- Iocetamic Acid (*Cholebrine*)

4. Radioactive Iodine:

Millicurie amounts of ^{131}I (*Iodotope I-131*) are used for thyroid ablation in the management of hyperthyroidism. ^{131}I is concentrated selectively by the thyroid gland. The ablative effect is exerted primarily through β particle emissions, which destroy thyroid tissue.

5. Lithium carbonate:

Lithium inhibits thyroidal incorporation of I^- into Tg, as well as the secretion of thyroid hormones, but it does not inhibit the activity of the Na^+–I^- symporter or the accumulation of I^- within the thyroid.**(60)**

- **Thyroid Surgery:**

A **thyroidectomy** is an operation that involves the surgical removal of all or part of the thyroid gland. Thyroidectomy is an effective method of therapy for patients in whom RAI or thioamides are contraindicated; for those with large goitres, causing cosmetic disfigurement; for those with suspected malignancies; and for selected pregnant and paediatric patients. The ideal surgical end point is a 3 to 8gm remnant of thyroid tissue, left after surgery that produces neither a recurrence of the thyrotoxicosis nor hypothyroidism.

A subtotal thyroidectomy is the most popular form of surgery performed for hyperthyroidism because it offers the best chance of euthyroidism. Others advocate a total thyroidectomy, for which hypothyroidism is a recognised side effect of surgery for which levothyroxine replacement will be needed lifelong.**(61)**

1.16. SPECIAL TREATMENT CONSIDERATIONS

❖ **Exophthalmos**

Exophthalmos is the bulging of the eye anteriorly of the orbit. If left untreated exophthalmos can cause the eyelids to fail to close during sleep leading to corneal dryness and damage. Another possible complication is a form of redness or irritation called **superior limbic kerato conjunctivitis,** where the area above the cornea becomes inflamed as a result of increased friction during blinking.

Various degrees of the following signs and symptoms occur:

- Edema and swelling of the lids and periorbital tissue, causing chemosis, excessive tearing, photophobia and conjunctivitis.
- Proptosis, which produces a wide-eyed staring expression. Cornea scarring and ulceration can occur if the proptosis causes the lid to remain open, exposing and drying out the eye during sleep.
- Limitation of the extraocular eye movements from paralysis of the extraocular muscles.
- Blindness may occur from venous congestion and haemorrhage of the retina and optic nerve. **(62)**

TREATMENT
• Some clinicians favour thyroid ablation with either RAI or surgery to remove the gland. • The grittiness of the eye can be treated with hypromellose eye drops • severe complications from proptosis, diplopia or visual failure should be treated with high dose prednisolone(60-120md/day) until symptoms resolve

TABLE NO: 2.2

❖ **Thyroid Storm**

It's an exaggerated form of thyrotoxicosis. It's a medical emergency characterized by

a) Acute onset of high fever
b) Cardiovascular symptoms like tachycardia, shock, tachyapnea, arrhythmia and congestive heart failure.
c) Gastro intestinal symptoms like diarrhoea and vomiting
d) CNS symptoms like agitation and psychosis.[63]

TREATMENT
a) Support of vital functions with sedation, O_2, fluids, antipyretics, treatment of infection, electrolyte balance. b) Use of thioamide and iodides to block synthesis and release of hormones. c) Blockage of metabolic effects by using propranolol d) Remove circulating hormone by plasmapheresis, exchange transfusion and dialysis when response to drug is failed.

TABLE NO: 2.3

❖ **Atrial Fibrialltion**

Hyperthyroidism can cause new onset or worsening of atrail fibrialltion and congestive heart failure. The atrial fibrialltion is often difficult to control until euthyroidism is achieved.

A combination of medications including β blocker, calcium channel blocker and a large dose of digoxin is required to slow the heart. Anticoagulant with caumadin is recommended in those with valvular disease and heart disease because of high incidence of emboli.[64]

❖ **Neonatal thyrotoxicosis**

Neonatal thyrotoxicosis results from stimulation of the foetal thyroid by transplacental passage of thyroid receptor stimulating antibodies from the maternal circulation. The infants require supportive measures including sedation, cooling, electrolyte replacement and treatment with thioamides, iodides or β- blockers.[65]

1.17. HERBAL THERAPY FOR HYPER THYROIDISM

Four herbs are commonly suggested by Western herbalists, other practitioners of complementary and alternative medicine and naturopathic medical textbooks for treating hyperthyroidism.[66] Three herbs appear to have effects on thyroid hormone-- lemon balm (Melissa officinalis, Lamiaceae), bugleweed (Lycopus virginicus, Lamiaceae), and gromwell (Lithospermum officinale, Boraginaceae); and one appears to reduce secondary symptoms of hyperthyroidism (heart palpitations and tachycardia), motherwort (Leonurus cardiaca, Lamiaceae).[67]

- **Bugleweed (Lycopus virginicus) and Gypsywort (Lycopus europaeus)**

These two closely related herbs have been used traditionally for the treatment of overactive thyroid conditions. Lycopus is naturalised in some wet areas of NZ and Australia. Lycopus appears to have several mechanisms of action[68]

- Inhibition of receptor binding of TSH and thyroid auto-antibodies to TSH receptors.
- Inhibition of iodine metabolism and release of thyroid hormone, by cyclic AMP inhibition at thyroid membrane**.**
- Oral doses of Lycopus inhibit the conversion of T4 to T3 in the peripheral tissues.

Hence, Lycopus is an essential herb to use in people with hyperthyroidism.[69]

- **Lemon balm (Melissa officinalis)**

Lemon balm is another very useful and safe herb to use in hyperthyroidism. Apart from its antithyroid actions, it also has a nervine relaxant effect which can be useful for the anxiety/irritability that often accompanies hyperthyroidism.

Possible mechanisms of action:

- Inhibits the binding of TSH and thyroid auto-antibodies to TSH receptors.
- Inhibition of conversion of T4 to T3 by blocking the enzyme responsible for this conversion.[70]

- **Motherwort (Leonorus cardiac)**

Motherwort is traditionally known as a heart tonic and uterine stimulant. Motherwort can be beneficial for treating some of the symptoms of hyperthyroidism such as anxiety, palpitations and increased heart rate.[71,72]

- **Gromwell (Lithospermum officinal)**

Gromwell is perhaps best known for its anti-gonadotrophic actions. It does appear to have antithyroid actions similar to lemon balm, by inhibiting the binding of TSH and thyroid auto-antibodies to TSH receptors.

- **Self-heal (Prunella vulgaris)**

Self heal is one of the richest sources of rosmarinic acid (5%). This compound can exert antithyroid activity after oxidation. Theoretically, therefore, self-heal may have some anti-thyroid activity[73]

Other Herb

- Emblica officinalis [74]

REVIEW OF LITERATURE

Chapter -2

2.1. REVIEW OF LITERATURE

Salman khan M, et al., (2013) investigated chemotherapeutic potential of Boerhaavia diffusa linked from ancient time to the present with the scope in future. Furthermore a recent update on mechanistic approaches of B. diffusa has also been discussed. Based on antioxidant & antdiabetic characteristic it is hypothesized that Boerhaavia diffusa might exhibit antiglycating properties as well.[(75)]

Kanjoormana Aryan Manu, et al.,(2009) evaluated Immunomodulatory activities of punarnavine,an alkaloid from B. diffusa using Balb/C mice. Punarnavine enhanced proliferation of splenocytes, thymocytes and bone marrow cells both in presence and absence of specific mitogens in vitro and in vivo. More over administration of Punarnavine significantly reduced the LPS induced elevated levels of pro inflammatory cytokines such as TNF-α, IL-1β and IL-6 in mice. [(76)]

Mandeep kaur, et al.,(2009) investigated the methanolic extract of Boerhaavia diffusa roots & its different fraction including liriodendrin rich fraction for exploring the possible role of liriodendrinrich in its anticonvulsant activity. These finding concluded that observed anticonvulsant activity was due to calcium channel antagonistic action as this activity was retained only in liodendrin rich fraction which posse's significant anticonvulsant activity of liriodendrin in BAY K-8644 induced seizures. [(77)]

Sandhya.k, et al., (2010) had conducted a comparative study of hydro alcoholic extract and poly herbal formulation of Boerhaavia diffusa for their anti stress activity using cold restrainst stress model.Due to cold restrain stress there was imbalance in the level of biochemical parameter like glucose, triglycerides, cholesterol, SGOT, SGPT which were near normalized following the administration of HEBD & PHF-09. HEBD and PHF-09 were found to have comparable anti stress activity. [(78)]

Surendar. k. Pareta et al.,(2011) found out the effects of pre treatment of aqueous extract of Boerhaavia diffusa root(200-400 mg/kg /day) in repeated dose acetaminophen nephrotoxic rats for 14 days. Acetaminophen administration characterized by significant increase in Blood urea nitrogen (BUN), serum creatinine and increased level of kidney malondialdehyde prolein thiol, along with depletion of SOD, CAT, GPX and GSH. .Histopathological changes showed significant structural damage to kidney. The result suggest that Boerhaavia diffusa has the potential in preventing the acetaminophen induced Nephrotoxicity. [(79)]

Ramachandran. y.L, et al.,(2011) evaluated the hepatoprotective properties of petroleum ether extract, Methanolic extract and isolated compound of B. diffusa & A. lanata against carbon tetra chloride induced hepatic damage in rats. This study reveals that different dose of plant extract offer significant protection of serum test and liver histology. [(80)]

Shisode. k.s, et al.,(2011) had studied that different extract of roots of Boerhaavia diffusa for invitro antioxidant activities & phytochemical screening. Among these there extract, ethanolic extract had shown better antioxidant activity & phytochemical screening revealed the presence of carbohydrate saponins, proteins, flavonoids, steroids, fats & alkaloid. [(81)]

Gopal. T.k, et al., (2010) evaluated invitro antioxidant activities of chloroform, ethanol & ethyl acetate fraction of B. diffusa.L which might have improved it's hepatoprotective action. The extract found to have significant Nitric oxide and DPPH radical scavenging activity. The result suggest that roots of Boerhaavia diffusa were found to reveal antioxidant potential which support the use of plant in traditional medicine. [(82)]

Apurba sarker Apu. et al., (2012) investigated the bioactivities of crude n-hexane, ethyl acetate and methanolic extract of aerial parts of the Boerhaavia diffusa linn and its phytochemical analysis. Methanolic extracts showed higher anti oxidant, thrombolytic activity and less cytotoxic activity than that of n-hexane & ethyl acetate extract of Boerhaavia diffusa. All the extract showed significant inhibitory activity aqainst candida albicans at a concentration of 1000µg/disc. These findings suggest that plant could be important source of medicinally important natural compound. [(83)]

Shukla Anamika and Gupta Rakesh kumar, (2011) studied the effects of aqueous extract of Boerhaavia diffusa roots and leaves on blood sugar level is Alloxan induced diabetic rats. These studies conclude that aqueous extract of B. diffusa have shown hypoglycemic effect may be due to presence of glycosides, flavonoids, tannins and saponin in the extract.[84]

Suralkas A.A et al.,(2012) investigated antihistamine activity of ethanolic extract of Boerhaavia diffusa linn roots using isolated goat tracheal chain and histamine induced bronchoconstriction in Guinea pig Boerhaavia diffusa significantly inhibited dose dependent contraction of goat tracheal chain produced by histamine and also showed significant protection by prolonging preconvulsion dyspnoea time in guinea pigs.Thus Boerhaavia diffusa showed antihistaminic and bronchodilating activity against histamine and hence posses potential role in treatment of asthma. [85]

Surendran. k, et al., (2010) evaluated anti urolithiatic activity of Boerhaavia diffusa lina root aqueous extract and rationalize it's use in treating renal stone. The lithogenic treatment causes weight loss, hyperoxalurea and impairment of renal function. Boerhaavia diffusa linn causes diuresis and hasten the process of dissolving crystals and helps in mechanical expulsion of stones and improve the renal function by removing the waste product and decrease oxalate excretion by interfering with metabolism. Results of this study indicate Boerhaavia diffusa lina posses antiurolithiatic that possibly mediated through diuretic and hypo-oxaluric effects. [86]

Mahesh, A.R, et al.,(2012) had conducted a detailed study on Boerhaavia diffusa for it is medicinal importance. Various phytochemical ,pharmacological ,experimental and clinical investigation are done on Boerhaavia diffusa. This include evidence based over view of pharmacological, phytochemical properties of aerial parts & the roots of Boerhaavia diffusa which may be helpful to establish a standard natural drug for further studies. [87]

Meena, A.K, et al., (2010) investigated the standardized and phytochemically evaluated aqueous and hydroalcoholic extracts of Boerhaavia diffusa. It involve pharmacognostical examination of morphological and microscopical characters and phytochemical investigation of Boerhaavia diffusa including determination of loss on drying, ash values,

TLC and extractive values. The qualitative chemical examination revealed the presence of various phytoconstituents like carbohydrate, ,saponins,phenolic compound and mucilage in the extract. [88]

Babita Aqrawal,et al., (2011) have investigated a review on it is phytochemical and pharmacological profile Phytochemical studies had shown the presence of rich source of alkaloids, steroids and flavones. pharmacological research explains hepatoprotective, diuretic, anti inflammatory ,anti-stress and immunomodulation anti fertility, antimicrobial, antiviral, and insecticidal activities. In conclusion Boerhaavia diffusa contain biologically active compounds that may serve as candidate for new drugs in the treatment and prevention of human live stock diseases. [89]

Goyal.B.M et al ., (2010) analyzed an overview of pharmacological potential of Boerhaavia diffusa. It covers various physiology, pathology of disease and their therapies. This article includes evidence based information regarding pharmacological activity of this plant. It has many ethanobotanical users and is medicinally used in the traditional Ayurvedic system. [90]

Bhavin, A, et al.,(2013) investigated the effect of hydro alcholic extract of roots of Boerhaavia diffusa in experimental Beningn prostatic hyperplasia in rats. Body weight, prostate weight, bladder weight and serum testosterone were measured and histological studies were carried out. The result suggested that treatment with Boerhaavia diffusa may improve symptoms of disease and inhibit the increased prostrate sign. Invitro study implies that herbal extract had a beneficial effect on prostatic smooth muscles which relieve the urinary symptom and disease. [91]

Krishna murti, et al., (2001) evaluated antidiabetic activity of ethanolic extract of roots of Boerhaavia diffusa against streptozocin induced experimental rats. Blood glucose level were determined on 0, 7^{th}, 14, & 21^{st} day after oral administration. The effect of ethanolic extract of B.diffusa on serum lipid profile like total cholesterol, triglycerides, LDl,VLDL, HDL were also measured in diabetic and non diabetic rats.The ethanolic extract of Boerhaavia diffusa was found to reduce blood sugar level in streptozocin induced diabetic rats. There was significant reduction in total cholesterol,LDL, VLDL, &

improvement in HDL cholesterol in diabetic rats. The results indicated that Boerhaavia diffusa posses a hypoglyceamic & antihyperlipideamic effect. [92]

Ajmire. P.V, etal., (2011) had conducted study of alcoholic & aqueous extract of whole plant of Boerhaavia diffusa against DMNO induced liver cirrhosis in rat's model. The activity was assessed using ILS, histopathological studies of liver, biochemical & heamatological studies. EEBD & AEBD shows significant increase in survival time, a decrease in cirrhotic nodules. The biochemical & heamatological parameter were also corrected by EEBD & AEBD in DMN induced rats. However out of these two extract, EEBD shows maximum anti cirrhotic effect than AEBD. [93]

Venkatesh. P, et al (2012) evaluated analgesic & antipyretic activity of various doses of alcoholic extracts of stem & leaves of Boerhaavia diffusa & leaves of Anisochilus carnosus. Tail immersion method & Hot plate in mice were studied for analgesic activity. Alcoholic extract of Boerhaavia diffusa had shown significant analgesic & antipyretic activity. [94]

Mohammed Khalid, et al., (2012) had studied pharmacological evalution and qualitative analysis of Boerhaavia diffusa L. root. Various parameters like macroscopy, microscopy, fluorescence analysis as well as extraction value and qualitative phytochemical screening of different extraction were studied. The major components of extractions like total phenolic, total flavonoids were also estimated. [95]

Venkatesh. P, et al., (2012) evaluated a study on alcoholic extract of stem and leaves of Boerhaavia diffusa and leaves of Anisochilus carnosus on ccl4 induced hepatotoxicity in rats. Different dose levels administered. Biochemical parameters of liver like SGOT, SGPT, serum alkaline phosphatase, total and direct serum bilurubin were determined. It was concluded that the alcoholic extract of AEBD and AEAC possess hepato protective activity against ccl4 induced hepatotoxicity in rats. [96]

SCOPE OF THE PRESENT WORK

CHAPTER-3

3.1. SCOPE OF THE PRESENT WORK

The last couple of decades have seen a tremendous increase in interest in the biological properties of natural products as a means to identify novel compounds that could have potential in clinical medicine. To that end, flavonoids and flavonoid-like compounds percolate to the top due to their presence in diet constituents and reported beneficial effects on diverse biological processes and disease conditions. It has previously been reported that the consumption of flavonoids and some phenolic acids by experimental animals induced enlargement and histological changes in the thyroid gland. Ethanopharmacological studies on such herb, medicinally important plants continue to interest investigators throughout the world. One such plant, *Boerhaavia diffusa* linn invites attention of the researchers worldwide for its pharmacological activities.

The present investigation was undertaken to study the effect of the potential anthithyroid activity of Hydro alcoholic extract of *Boerhaavia diffusa* on L-thyroxine induced hyperthyroidism in wistar rats. Hydro alcoholic extract of *Boerhaavia diffusa* contain several medicinally active compounds including polyphenols(stilbenes and flavanoids), tannins and anthocyanins. The different parts of the plant *Boerhaavia diffusa* has been used in different system of traditional medication for the treatment of diseases and ailments of human beings. The effects produced by the extract on different parameters were compared with propyl thio uracil.

AIM OF THE WORK

CHAPTER-4

AIM

To identify a plant with potential antithyroid activity and to compare the effect of Hydro alcoholic extract of *Boerhaavia diffusa* (HAEBD) on L-thyroxine induced hyperthyroidism in rats with the standard drug propylthiouracil.

OBJECTIVES

- ✓ Evaluation of antithyroid activity of Hydro alcoholic extract of *Boerhaavia diffusa* against l-thyroxine induced hyperthyroid model.
- ✓ Compare antithyroid potential with propylthiouracil.

PLAN OF WORK

- ➢ Collection of plant material and preparation of plant extract.
- ➢ Evaluation of anti-thyroid activity and their effects on the thyroid hormones.
  a. Selection, grouping and acclimatization of the animals.
  b. Induction of hyperthyroidism in rats by using l-thyroxine
  c. Treatment protocol
  d. Evaluation of biological parameters
  e. Histopathological examination of thyroid gland of animals

PLANT PROFILE

CHAPTER-5

PLANT PROFILE

Figure no : 5 *BOERHAAVIA DIFFUSA*

Figure no : 6 ROOT OF *BOERHAAVIA DIFFUSA*

SCIENTIFIC CLASSIFICATION:

SCIENTIFIC NAME : *Boerhaavia diffusa* Linn. Syn. *B. repens; B. repens* var. *diffusa*
FAMILY : Nyctaginaceae
FAMILY NAME : Hog weed, Horse Pursl

TAXONOMICAL CLASSIFICATION:

KINGDOM : Plantae
FAMILY : Nyctaginaceae
DIVISION : Magnoliophyta
CLASS : Magnoliopsida
ORDER : Caryophyllales
GENUS : Boerhaavia
SPECIES : *Boerhaavia diffusa*

COMMON NAMES:

Raktapunarnava, Shothaghni, Kathillaka, Kshudra, Varshabhu, Raktapushpa, Varshaketu, Shilatika.

VERNACULAR NAMES:

BENGALI	:	Raktapunarnava
ENGLISH	:	horse purslene, hog weed
HINDI	:	Gadapurna, Lalpunarnava
KANADA	:	Sanadika, Kommeberu, Komma
MALAYALAM	:	ChuvannaTazhutawa
TAMIL	:	Mukurattai (Shihappu)
TELUGU	:	Atikamamidi, Erragalijeru

GEOGRAPHICAL DISTRIBUTION:

Boerhaavia diffusa is also indigenous to India; it is found throughout the warmer parts of the country up to an altitude of 2000 m in the Himalayan region. The genus Boerhaavia has several species, and is distributed in the tropical, subtropical, and temperate regions of the world.[158]It is found in Australia, China, Pakistan, Sudan, Sri Lanka, Egypt, SouthAfrica, USA and in several countries of the Middle East. Out of the 40 species of this genus, 6 species are found in India – *B. diffusa, B. chinensis, B. erecta, B. repens, B.rependa*, and *B. rubicund.*[(97)]

ORIGIN AND HABITAT:

Boerhaavia diffusa is a perennial creeping weed, prostrate or ascending herb, up to 1 m long or more, havingThis is found throughout India. It grows up to an altitude of 70 centimeters especially during the rainy season. It has a large root system and produces yellow and white flowers. It can be found in many tropical and warm-climate countries.[(98)]

USEFUL PARTS:

Root, leaves&seeds,stem,flowers, fruits

DESCRIPTION:

Boerhaavia diffusa is a perennial creeping weed, prostrate or ascending herb, up to 1 m long or more, having spreading branches.

The roots are very variable diffusely branched low spreading or creeping herbaceous perennial with an elongated fusiform or tapering tap root. The roots are stout and fusiform with a woody.

The stems are numerous; 1-2 m long &the stem is prostrate, woody or succulent, cylindrical, often purplish, hairy, and thickened at the nodes.[(99)]

Leaves are simple, thick, fleshy, and hairy, arranged in unequal pairs, green and glabrous. The shape of the leaves varies considerably – ovate-oblong, round, or subcordate at the base and smooth above. The leaves are simple, opposite, short petiolate, exstipulate, unequal in each pair, 2.5-5 cm long by 1-4.5 cm wide, oblong or suborbicular, acute, obtuse or rounded at apex, cordate, rounded or truncate at base, entire or wavy along the margin, subfleshy, glabrous or sparingly hairy above, silvery white beneath, petioles 0.7-3 cm long, slender, deeply grooved above.[(100)]

The flowers are small, regular, sessile or subsessile, pale rose to pink, in irregular clusters of 4-10, small umbels on extra axillary peduncles.[(101)]

The fruits are very small, one seeded and enclosed in persistent lower half the perianth. The perianth is covered with sticky glandular hairs.

Part	A perennial herb from a fusiform root
Plant	*Boerhaavia diffusa*
Leaves	Opposite or sub-opposite, two of a node unequal, broadly ovate or sub-orbicular, obtuse to rounded or sub-cordate at the base.
Stem	Prostrate, decumbent or ascending, 4-10 cm long, rather slender, divaricately branched
Flowers	In pendunculate, glomerulate clusters arranged in slender, long stalked, axillary or terminal corymbs
Fruit	Ovoid or sub-ellipsoid, rounded above, slightly cuneate, below, broadly and bluntly 5-ribbed, very glandular throughout
Flowering and Fruiting	Throughout the year in Indian conditions

PHYTOCHEMICALS:

Boerhaavia diffusa contains a large number of phytoconstituents, namely flavonoids alkaloids, steroids, triterpenoids, lipids, lignins,carbohydrates proteins and glycoproteins .[102]

- Plant also includes a series of rotenoidsi.eboeravinones from roots of the plant viz., Boeravinone (A-F) [103-105]
- Punarnavoside, a phenolic glycoside, is reportedly present in roots.C-methyl flavone also has been isolated from *Boerhaavia diffusa* roots.[106]
- Two known lignans viz., liriodendrin and syringaresinol mono-β-D-glycoside isolated[107]
- Presence of a purine nucleoside hypoxanthine 9-L-arabinose[108] dihydroisofuroxanthone-borhavine[109], phytosterols[110] have been isolated from the plant.
- It contains about 0.04 % of alkaloids known as punarnavine and punernavoside, an antifibrinolytic agent.

- It also contains about 6% of potassium nitrate, an oily substance, and ursolic acid. [111]
- The seeds of this plant contain fatty acids and allantoin and the roots contain alkaloids.[112] The green stalk of the plant has also been reported to contain boerhavin and boerhavic acid.

EXPERIMENTAL DESIGN

CHAPTER-6

EXPERIMENTAL DESIGN

Experimental Models

For the study of anti-thyroid activity of HAEBD, an experiment model is selected in such a way that it would satisfy the following:

- The animal should develop hyperthyroidism rapidly
- Pathological changes in the site of induction should result from damage to the follicular cells of thyroid
- The symptoms should be ameliorated or prevented by a drug treatment effective in humans
- The drug tested must be administered orally
- Drug dosage should approximate the optimum therapeutic range for human, scaled the test animal weight.

Selection of Laboratory Animal

- Rats are the most commonly used animals in the study of thyroid disorders
- Animals such as sheep, cats, dogs ,rabbit and guinea pigs are also used
- In the present study rats have been used because, the thyroid hormone production and metabolism of rat resembles to that of humans which is believed to contribute to hyperthyroidism studies

Materials and Methods

Animals : Albino Wistar Rats (190-240gm)

Drugs : Hydro Alcoholic Extract of Boerhavia diffusa

: L-Thyroxine (T4) (Sigma,USA)

: Propylthiouracil (Macleods Pharmaceuticals Ltd)

The study was approved by Institutional Animal Ethics committee (IAEC) of K.M. College of Pharmacy, Uthangudi, Madurai, which is registered with Committee for the Purpose of Control and supervision of Experimental Animals (CPCSEA), Government of India,(Registration number and date of registration:661/02/c/CPCSEA & 19/07/2002.

SELECTION AND ACCLIMITIZATION OF ANIMALS

Adult male wistar rats (6week old) weighing 190–240 g were used in the experiments after allowing 15 days acclimatization. The animals were allocated four per polycarbonate cage in a temperature (20–25°C) and humidity (40–45%) controlled room. The light: dark cycle was 12 hr: 12 hr and normal rodent pellet diet and water were supplied during acclimatization, free to access.

INDUCTION OF HYPERTHYROIDISM

After acclimatization, hyperthyroidism was achieved by daily oral administration of L-Thyroxine (T4) (Sigma,USA) at a dose of 600µg/kg for 12 consecutive days according to the previous established method .[113]

Levothyroxine acts like the endogenous thyroid hormone thyroxine (T_4, a tetra-iodinated tyrosine derivative). It is a synthetic form of the thyroid hormone thyroxine, which is normally secreted by the follicular cells of the thyroid gland. L-thyroxine is commonly used to produce hyperthyroidism in experimental animals due to its ability to convert into t3, the active metabolite in the liver and kidney by the enzyme 5'-deiodinase.

Preparation of Drugs:

- Hydro-alcoholic extract of boherhavia diffusa is dissolved in sterile water.
- Propyl thiouracil tablets were weighed, powdered and triturated with saline.
- L-thyroxine tablets were weighed and dissolved in distilled water.

TREATMENT PROTOCOL

Animals were randomly divided into 5 groups of 6 rats each after 12 days L-thyroxine (T_4) treatments as follows

Group-1 Served as normal control received 10ml/kg of normal saline.

Group-2 Served as hyperthyroidoism control received distilled water orally for 15 days.

Group-3 Served as treatment control received 10mg/kg of propyl thiouracil(PTU),orally for 15 days.[114]

Group-4 Served as treatment control received 200mg/kg of Hydro Alcoholic Extract of Boerhaavia Diffusa orally for 15 days.

Group-5 Served as treatment control received 400mg/kg of Hydro Alcoholic Extract of Boerhaavia Diffusa orally for 15 days.[115]

After 12 days of L- thyroxine treatment, propyl thio uracil was injected intraperitoneally to G3 group in a volume of 5ml\kg dissolved in saline for 15 days to G3 group and plant extract of low dose and high dose administered to G4 and G5 group for 15 days. The dosages of BD extracts used in this study were selected based on the previous report, in which 400 mg/kg of BD extracts showed enough in vivo pharmacological effects in rats and propyl thio uracil 10 mg/kg was also selected based on the previous in vivo efficacy test on the L-THYROXINE(T_4)-induced hyperthyroidisms in rodents.. Equal volume of saline was subcutaneously treated in intact control rats instead of L-THYROXINE (T_4), and equal volume of distilled water was orally administered in intact and L-THYROXINE (T_4) control rats, instead of BD extracts or propyl thio uracil [116]

METHADOLOGY

After 15 days of treatment, the blood was collected from the retro-orbital plexus puncture of all groups of overnight fasted rats using micro capillary. The serum was separated for the estimation of thyroid hormones (TSH, T_3, and T4) and liver enzymes (AST, ALT).Then the animal was sacrificed by decapitation. The thyroid gland was immediately dissected out, washed in ice cold saline to remove the blood and stored in 10% formalin for histopathological studies. The liver was also separated and homogenised for the estimation of lipid peroxidation and antioxidant defense system.

FIG NO: 7

A- LARYNX, B-THYROID GLAND, C-SUB MANDIBULAR GLAND, D-TRACHEA

CHAPTER-7
PHARMACOLOGICAL EVALUATION

Estimation of Biochemical Parameters

- **Serum Thyroid Hormones**

6mL of blood samples were collected into evacuated tubes, and serum was separated by centrifugation at 3000 rpm for 10 min at 4◦C. Separated serum was stored at −70◦C before analysis. Serum levels of T3, T4, and thyroid-stimulating hormone (TSH) were analyzed by colorimetric competitive enzyme immunoassay using individual ELISA kit according to Subudhi et al. , respectively.[117]

- **Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)**

Serum AST and ALT concentrations were measured by automated blood analyzer according to previous method.[118]

- **Liver Lipid Peroxidation (LPO)**

Separated liver tissues were weighed and homogenized in ice-cold 0.01M Tris-HCl (pH 7.4), and then centrifuged, at 12,000 g for 15 min as described by Kavutcu et al.[119] The concentrations of liver LPO were determined by estimating malondialdehyde (MDA) using the thiobarbituric acid test at absorbance 525 nm, as nM of MDA/mg protein .[120]

- **Liver Antioxidant Defense Systems**

Prepared homogenates were mixed with 0.1mL of 25% trichloroacetic acid (Merck, CA, USA), and then centrifuged at 4,200 rpm for 40 min at 4◦C. Glutathione (GSH)[121] contents were measured at absorbance 412nm using 2-nitrobenzoic acid (Sigma,MO, USA).Decomposition of H2O2 in the presence of catalase was followed at 240nm [122]. Catalase activity was defined as the amount of enzyme required to decompose 1nM of H2O2 per minute, at 25◦C and pH 7.8. Results were expressed as U/mg protein. Measurements of SOD activities were made according to Sun et al.[123] SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitrotetrazolium blue to form formazan dye.SOD activity was then measured at 560nm by the degree of inhibition of this reaction, and was expressed as U/mg

protein. One unit of SOD enzymatic activity is equal to the amount of enzyme that diminishes the initial absorbance of nitroblue tetrazolium by 50% during 1min.

Histology

The sampled thyroid gland tissues were fixed in 10% neutral buffered formalin. After paraffin embedding, 3–4 μm serial sections were prepared. Representative sections were stained with hematoxylin and eosin (H&E) for an optical microscopy examination. Subsequently, the histological profiles of the organs were observed. The mean cross thickness of thyroid gland, thyroid follicle, and follicular lining epithelium were measured using an automated image analysis process.

Statistical Analysis

Numerical data are presented as mean± S.E.M. of six rats, the obtain data was analyzed using a one way ANOVA test followed by Newmann Keuls multiple range tests. Statistical analyses were conducted using graphpad version 3.1. P values < 0.05 were considered significantly different.

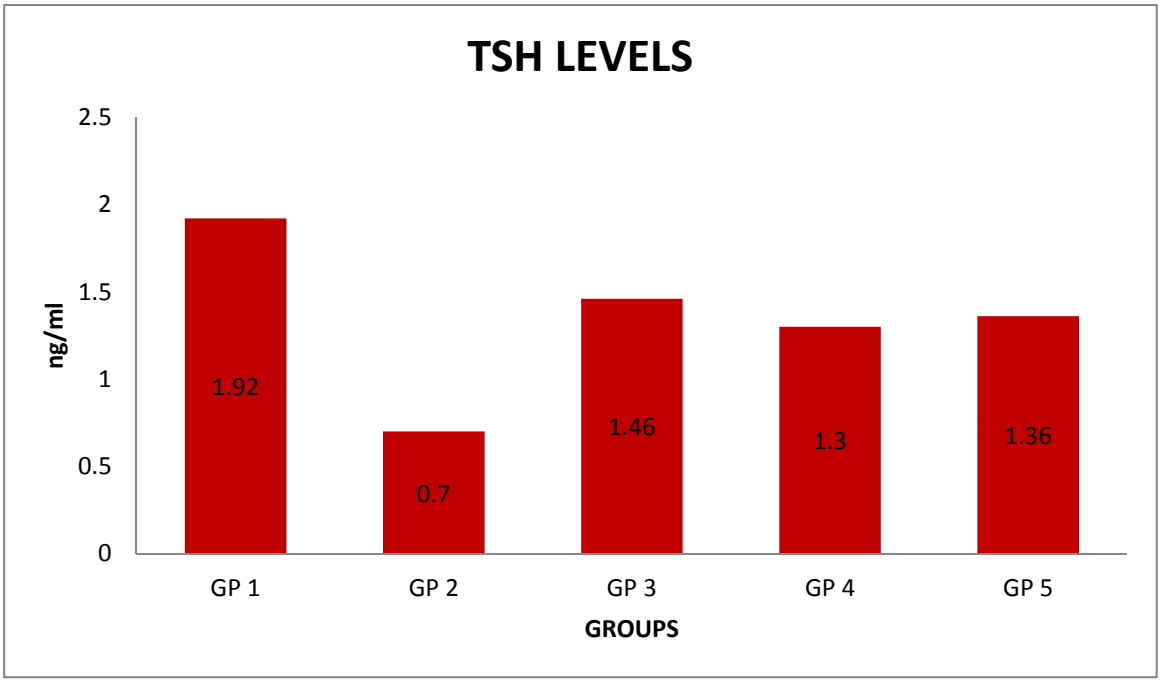
Table No: 3

SERUM THYROID HORMONE LEVELS IN THE L-THYROXINE (T_4) AND HAEBD TREATED RATS

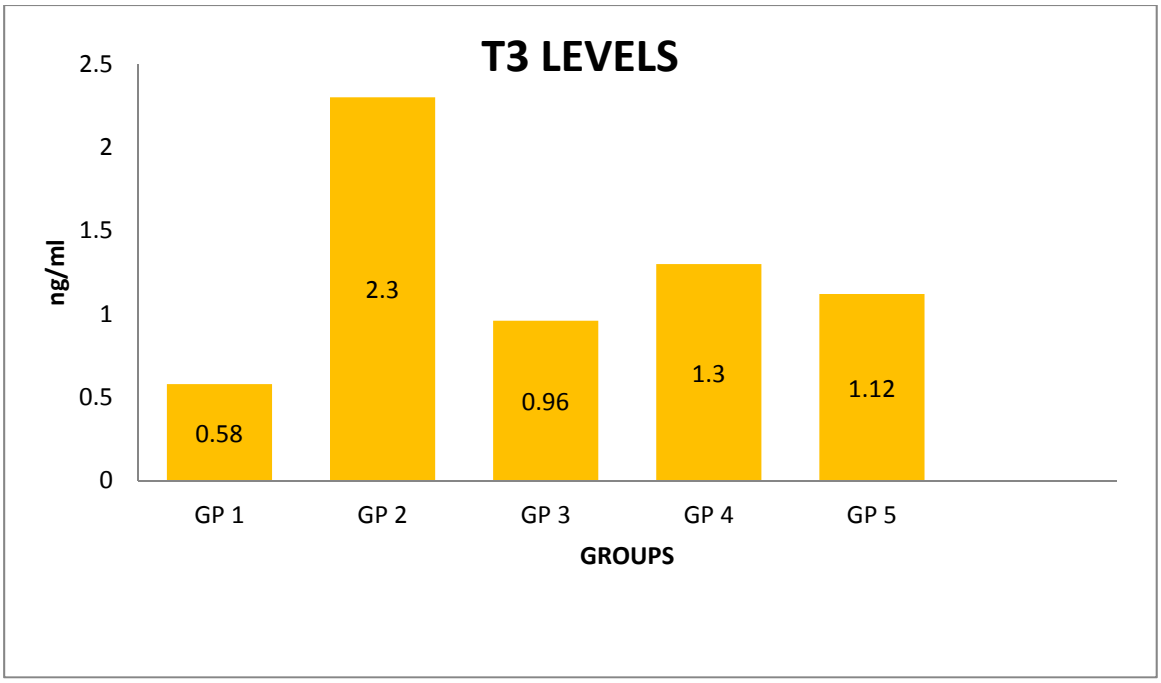
GROUPS	TSH(ng/ml)	T_3(ng/ml)	T_4 (µg/ml)
GP1	1.92±0.16	0.58±0.14	53.72±5.90
GP2	0.70±0.07*a	2.30±0.20*a	171.84±4.80*a
GP3	1.46±0.12*b	0.96±0.12*b	72.30±5.35*b
GP4	1.30±0.10*b	1.30±0.30*b	86.12±3.10*b
GP5	1.36±0.11*b	1.12±0.22*b	78.30±4.15*b

GP_1- Normal; **GP_2**- Hyper Control; **GP_3**- Standard Control(PTU 10mg/kg); **GP_4**- HAEBD (200mg/kg);**GP_5**- HAEBD (400mg/kg)

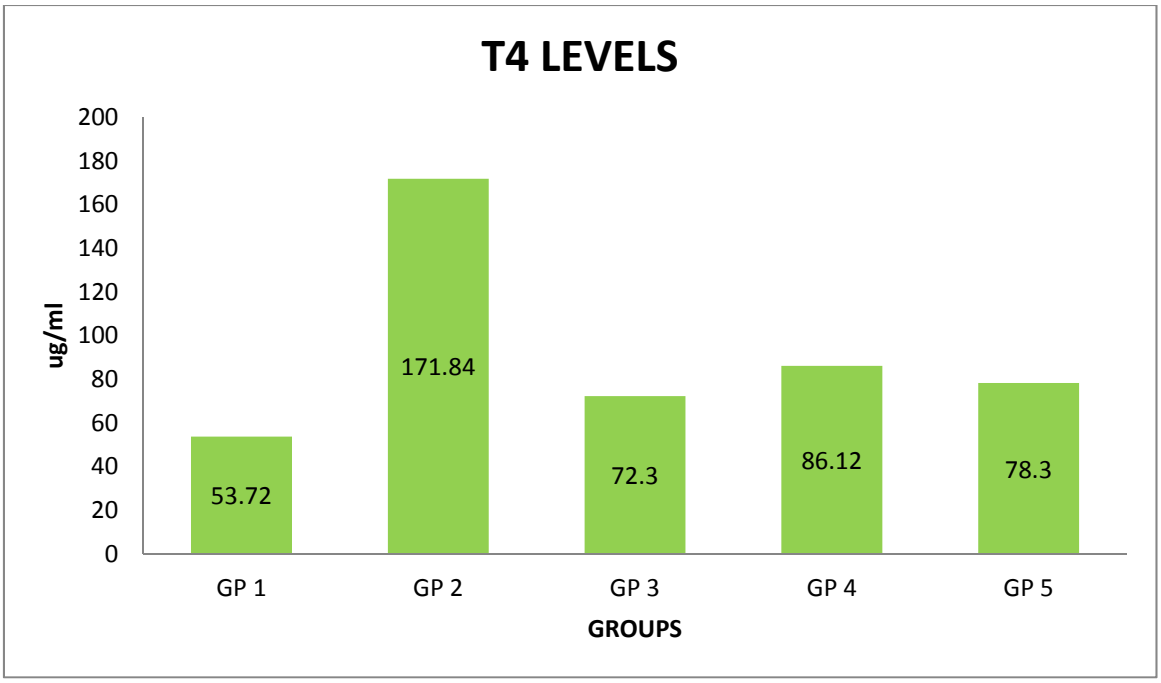
- All values are expressed as mean ± SEM for 6 animals in each group.
- **a – Values are significantly different from Normal control (G_1) at $P < 0.01$
- **b – Values are significantly different from hyperthyroid control (G_2) at $P < 0.01$



GRAPH 1. SERUM THYROID HORMONE LEVELS IN THE L-THYROXINE (TSH) AND HAEBD TREATED RATS



GRAPH 2. SERUM THYROID HORMONE LEVELS IN THE L-THYROXINE (T3) AND HAEBD TREATED RATS



GRAPH 3. SERUM THYROID HORMONE LEVELS IN THE L-THYROXINE (T4) AND HAEBD TREATED RATS

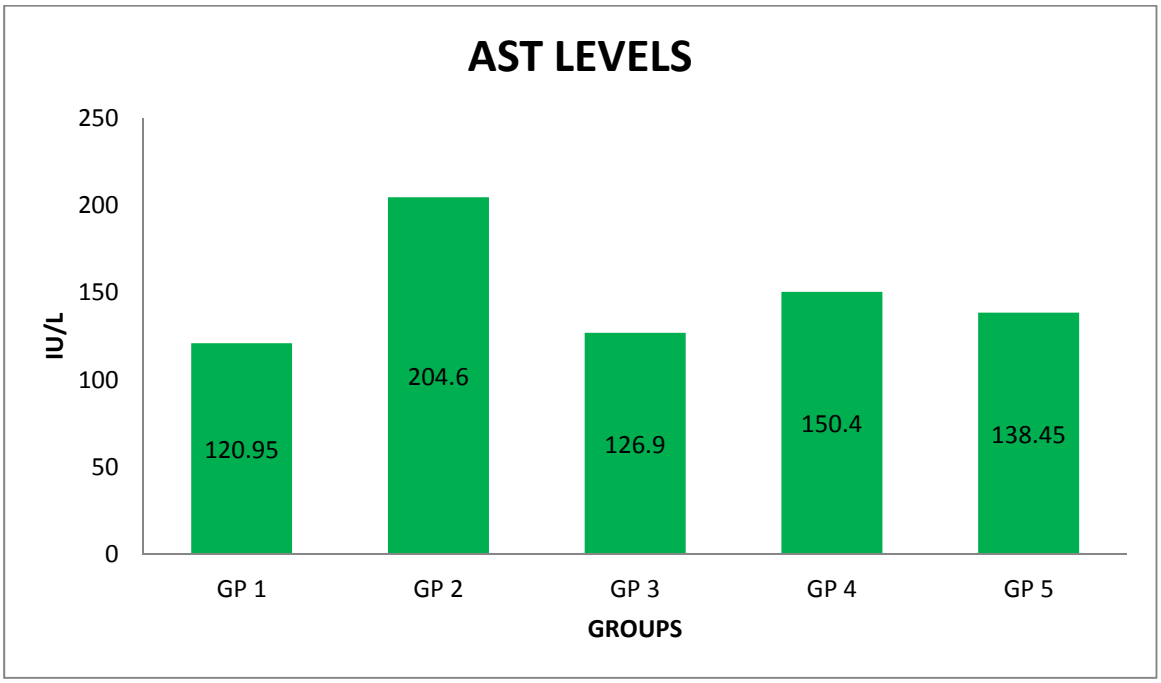
Table No: 4

EFFECT OF HAEBD ON SERUM LIVER ENZYMES LEVELS IN THE L-THYROXINE (T_4) AND HAEBD TREATED ANIMALS

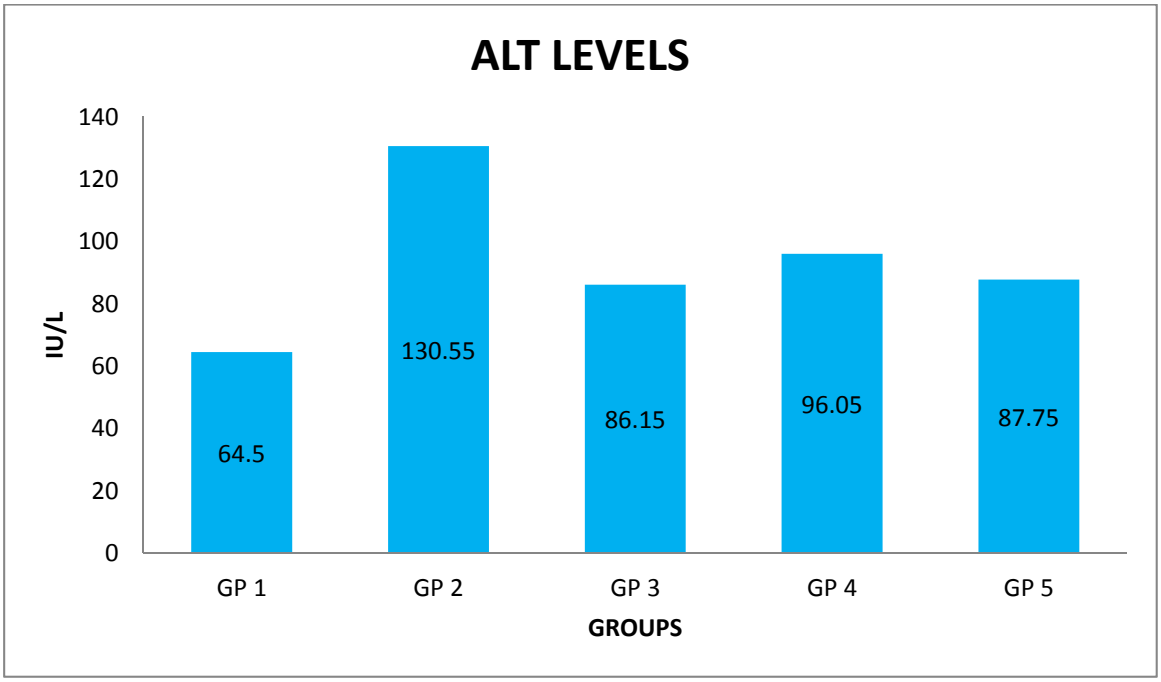
GROUPS	AST(IU/L)	ALT(IU/L)
GP1	120.95±5.20	64.5±3.28
GP2	204.60±8.80*a	130.55±3.75*a
GP3	126.90±5.45*b	86.15±3.50*b
GP4	150.40±3.60*b	96.05±4.85*b
GP5	138.45±3.68*b	87.75±3.90*b

GP_1- Normal; **GP_2**- Hyper Control; **GP_3**- Standard Control(PTU 10mg/kg); **GP_4**- HAEBD (200mg/kg);**GP_5**- HAEBD (400mg/kg)

- All values are expressed as mean ± SEM for 6 animals in each group.
- **a – Values are significantly different from Normal control (G_1) at $P < 0.01$
- **b – Values are significantly different from hyperthyroid control (G_2) at $P < 0.01$



GRAPH 4. EFFECT OF HAEBD ON SERUM LIVER ENZYMES LEVELS IN THE L-THYROXINE (AST) AND HAEBD TREATED ANIMALS



GRAPH 5. EFFECT OF HAEBD ON SERUM LIVER ENZYMES LEVELS IN THE L-THYROXINE (ALT) AND HAEBD TREATED ANIMALS

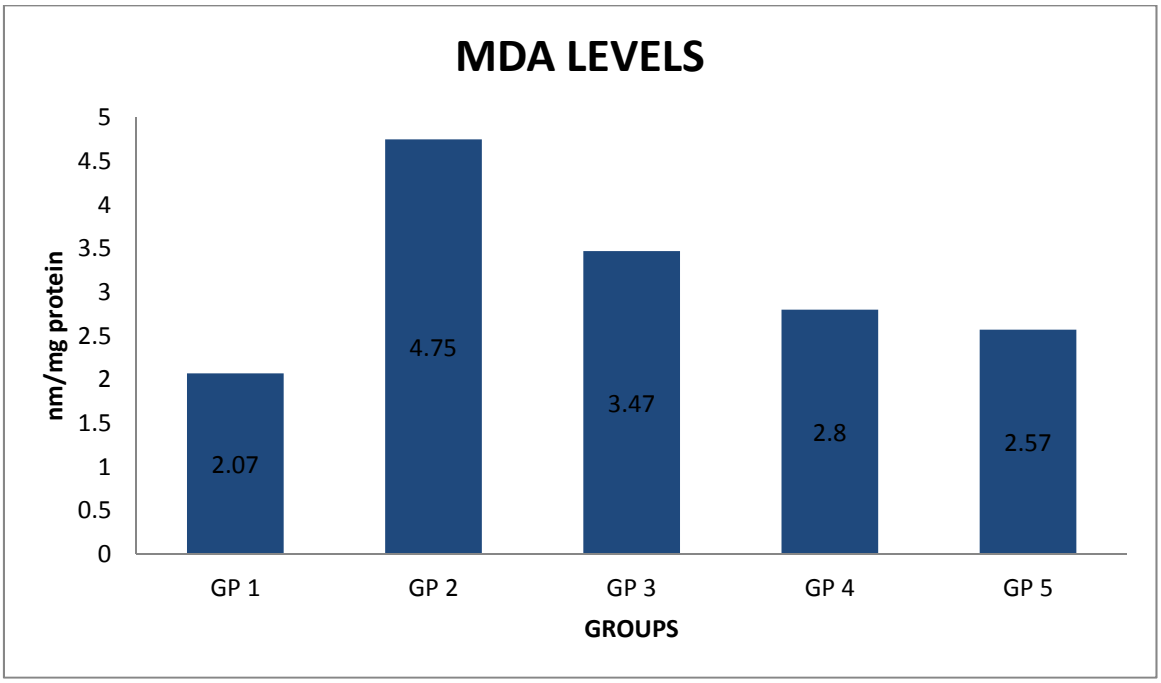
Table No: 5

LIVER LIPID PEROXIDATION AND ANTI OXIDANT DEFENCE SYSTEMS IN THE L-THYROXINE (T_4) AND HAEBD TREATED RATS

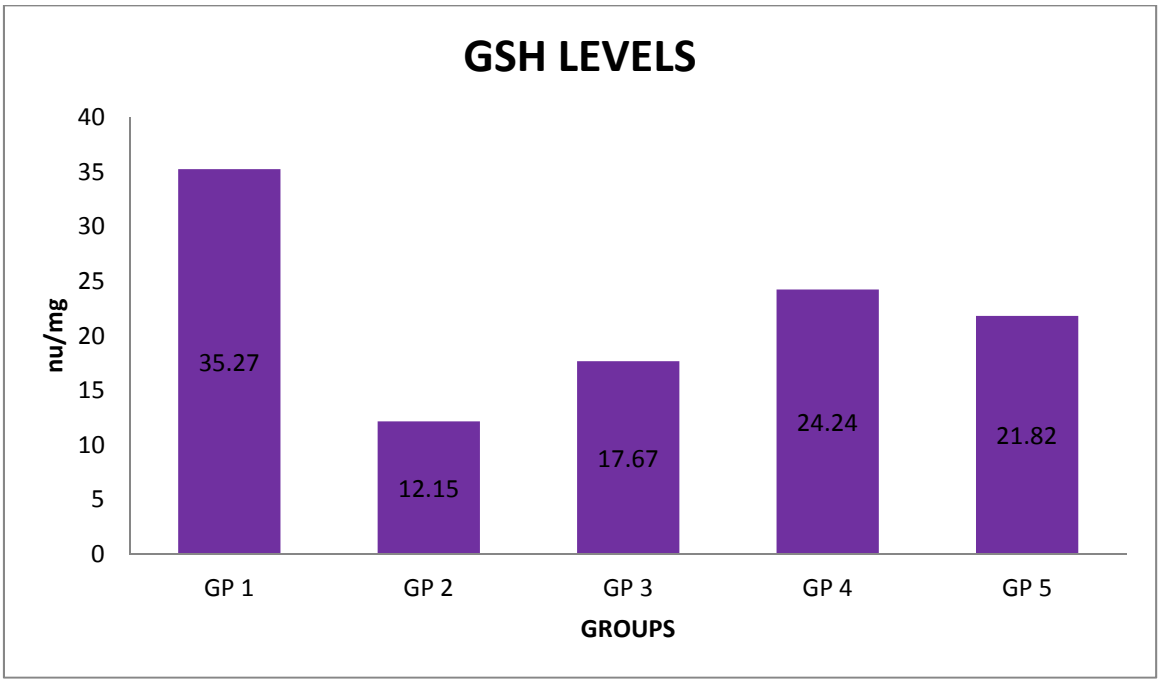
GROUPS	LIPID PEROXIDATION	ANTI OXIDANT DEFENCE SYSTEM		
	MDA(nm/mg protiein)	GSH (nμ/mg protein)	SOD U/mg protein	CATALASE U/mg protein
GP1	2.07±0.12	35.27±3.95	22.65±2.04	30.27±2.81
GP2	4.75±0.30*a	12.15±1.55*a	52.61±3.04*a	48.23±2.32*a
GP3	3.47±0.40*b	17.67±2.84*b	28.05±2.63*b	34.14±1.97*b
GP4	2.8±0.30*b	24.24±2.48*b	31.07±3.06*b	37.50±2.32*b
GP5	2.57±0.16*b	21.82±1.17*b	29.12±1.96*b	35.40±4.66*b

GP_1- Normal; **GP_2**- Hyper Control; **GP_3**- Standard Control(PTU 10mg/kg); **GP_4**- HAEBD (200mg/kg);**GP_5**- HAEBD (400mg/kg)

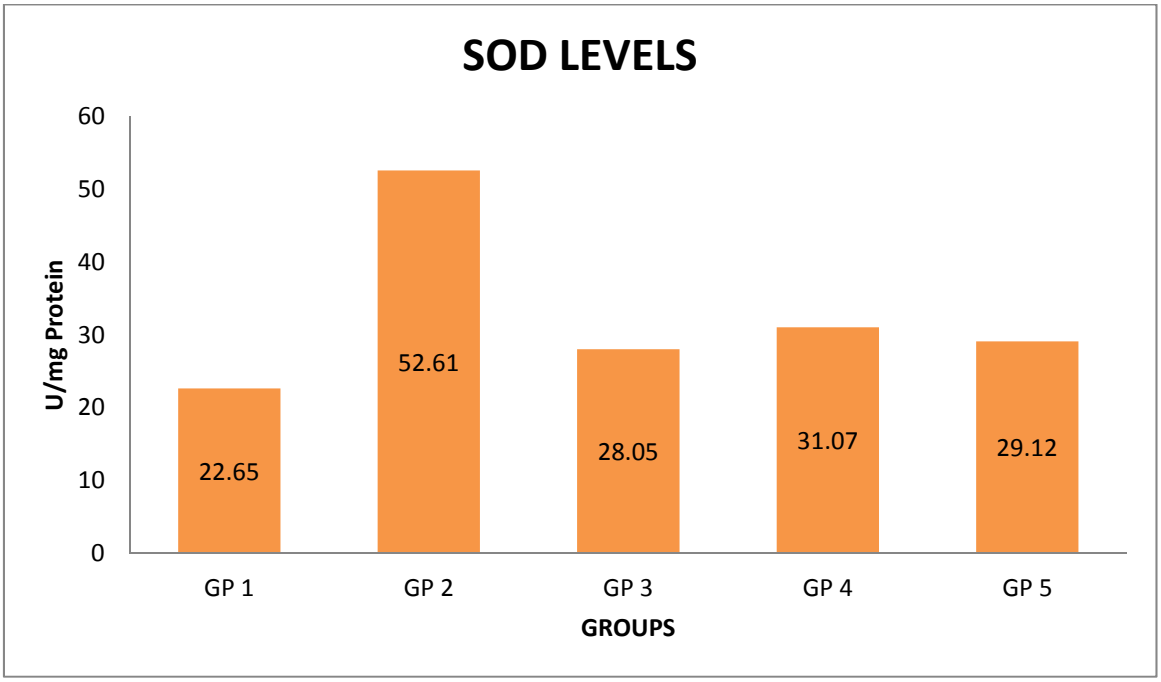
- All values are expressed as mean ± SEM for 6 animals in each group.
- **a – Values are significantly different from Normal control (G_1) at $P < 0.01$
- **b – Values are significantly different from hyperthyroid control (G_2) at $P < 0.01$



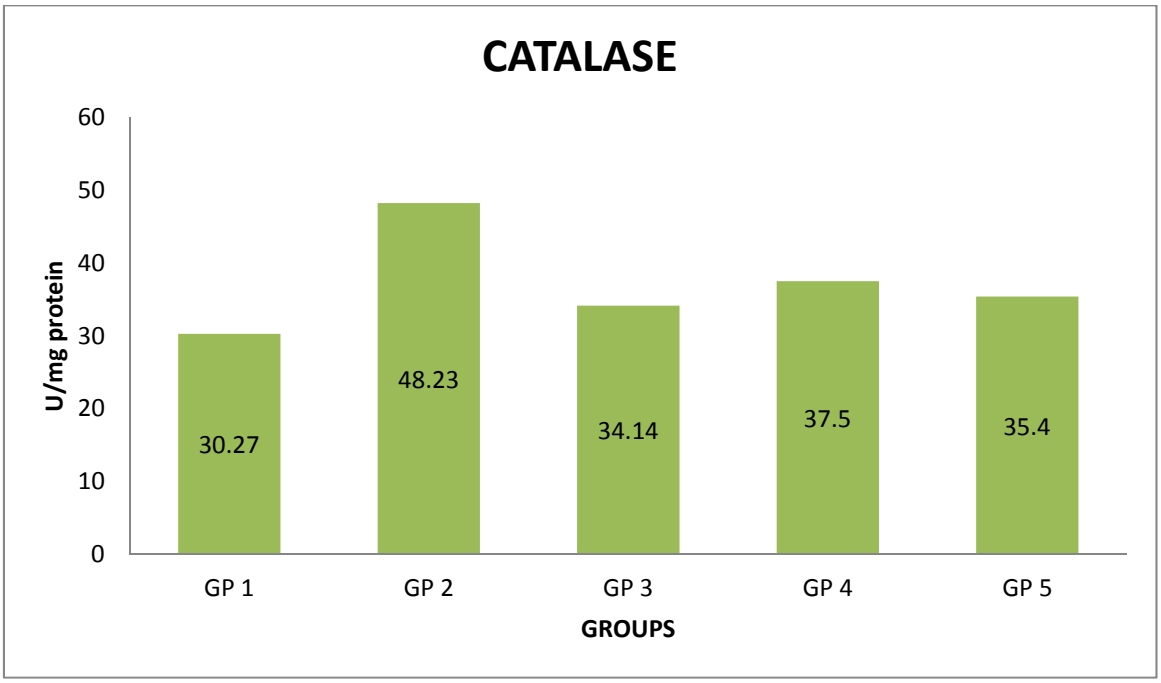
GRAPH 6. LIVER LIPID PEROXIDATION AND ANTI OXIDANT DEFENCE SYSTEMS IN THE L-THYROXINE (MDA) AND HAEBD TREATED RATS



GRAPH 7. LIVER LIPID PEROXIDATION AND ANTI OXIDANT DEFENCE SYSTEMS IN THE L-THYROXINE (GSH) AND HAEBD TREATED RATS



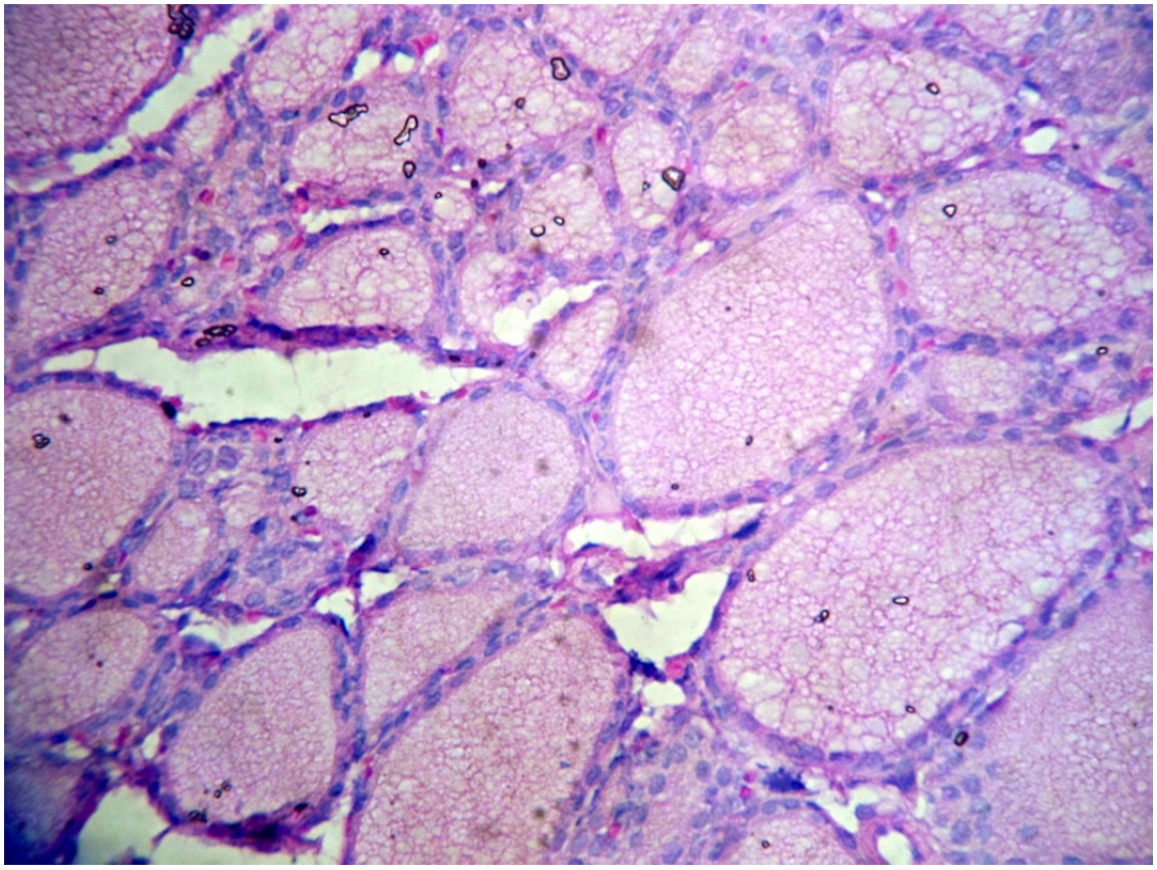
GRAPH 8. LIVER LIPID PEROXIDATION AND ANTI OXIDANT DEFENCE SYSTEMS IN THE L-THYROXINE (SOD) AND HAEBD TREATED RATS



GRAPH 9. LIVER LIPID PEROXIDATION AND ANTI OXIDANT DEFENCE SYSTEMS IN THE L-THYROXINE (CATALASE) AND HAEBD TREATED RATS

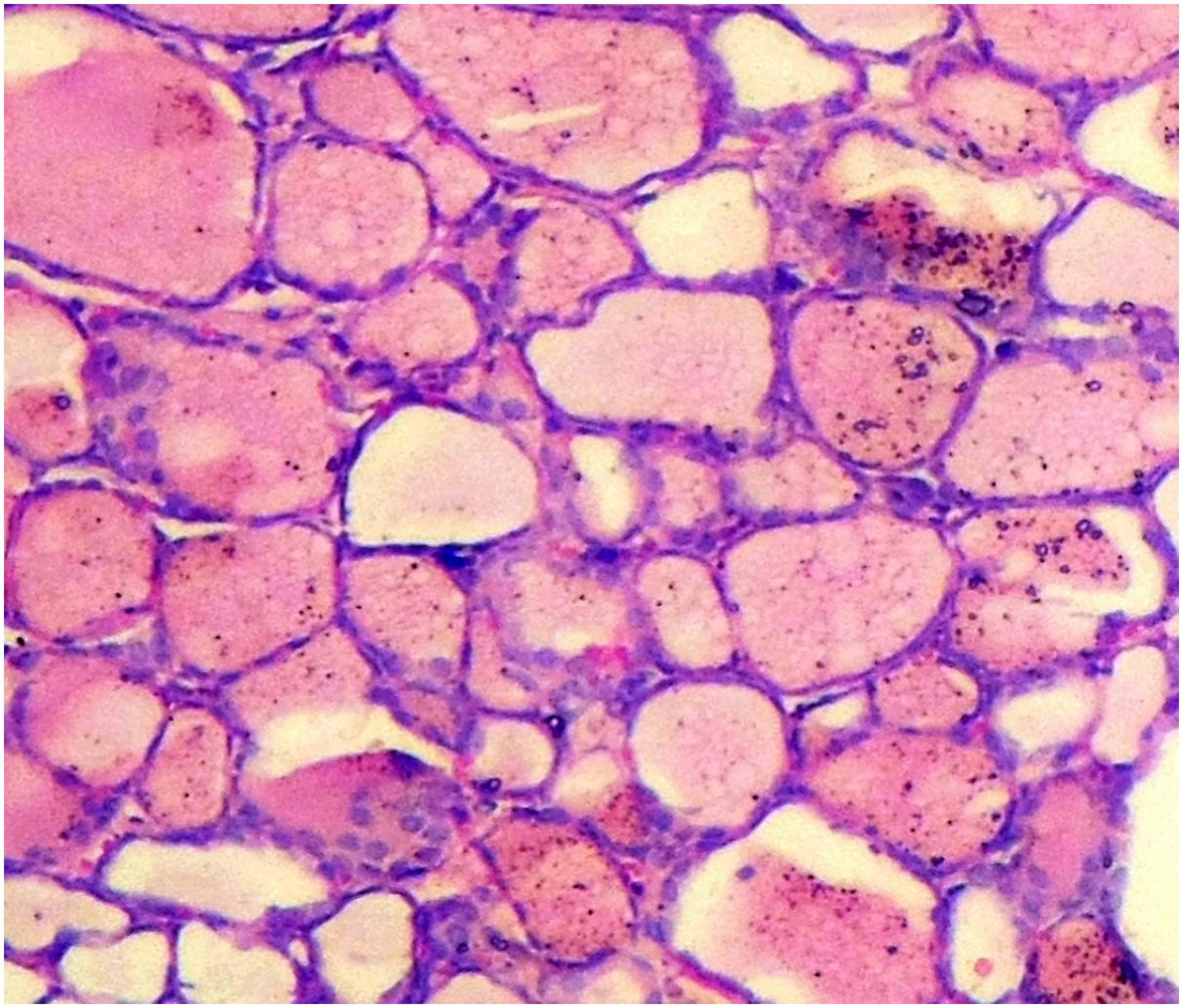
HISTOPATHOLOGICAL STUDIES

FIG NO: 8



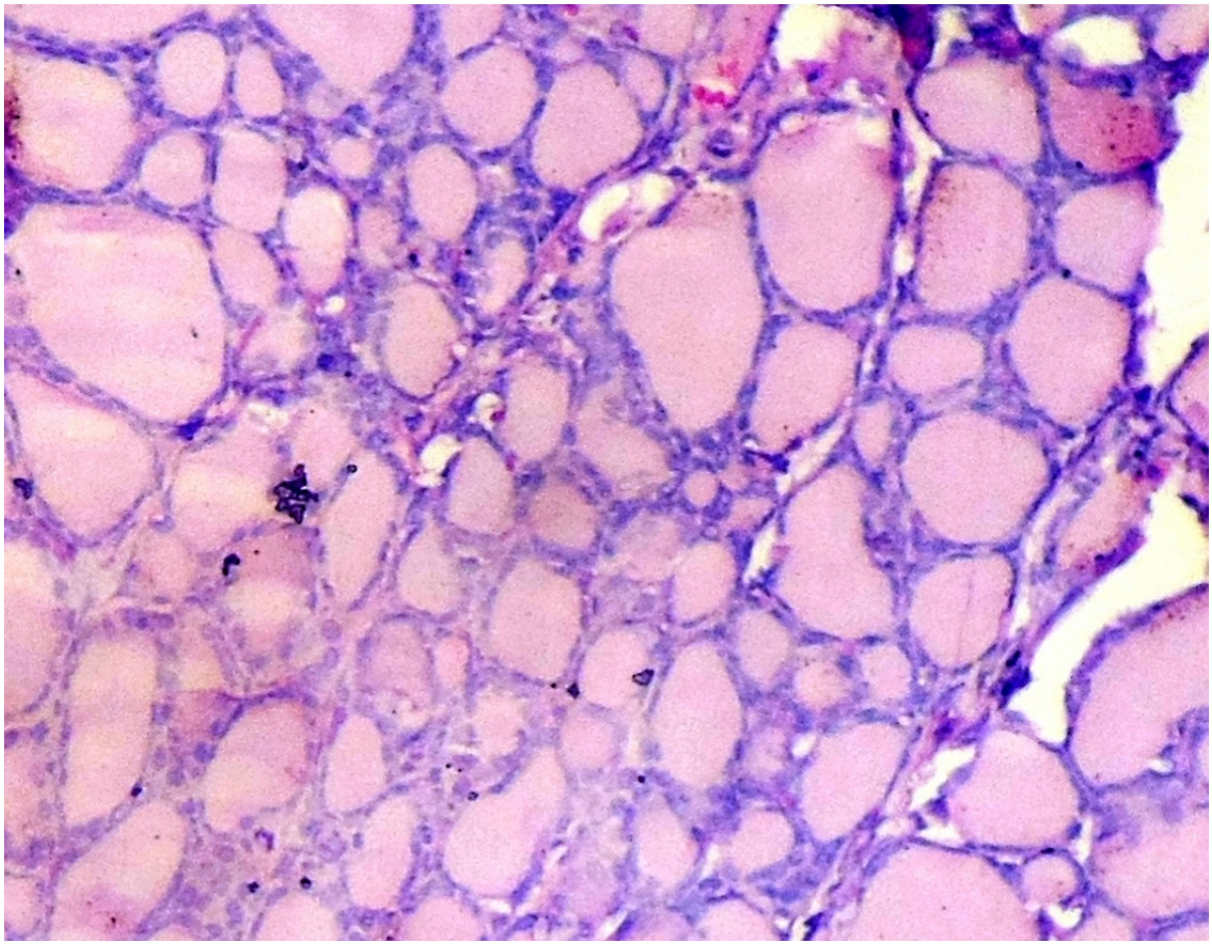
Thyroid gland section of GP1 rats (normal control) showing follicles lined by cuboidal epithelial cells filled with 60-70% colloid.

FIG NO: 9



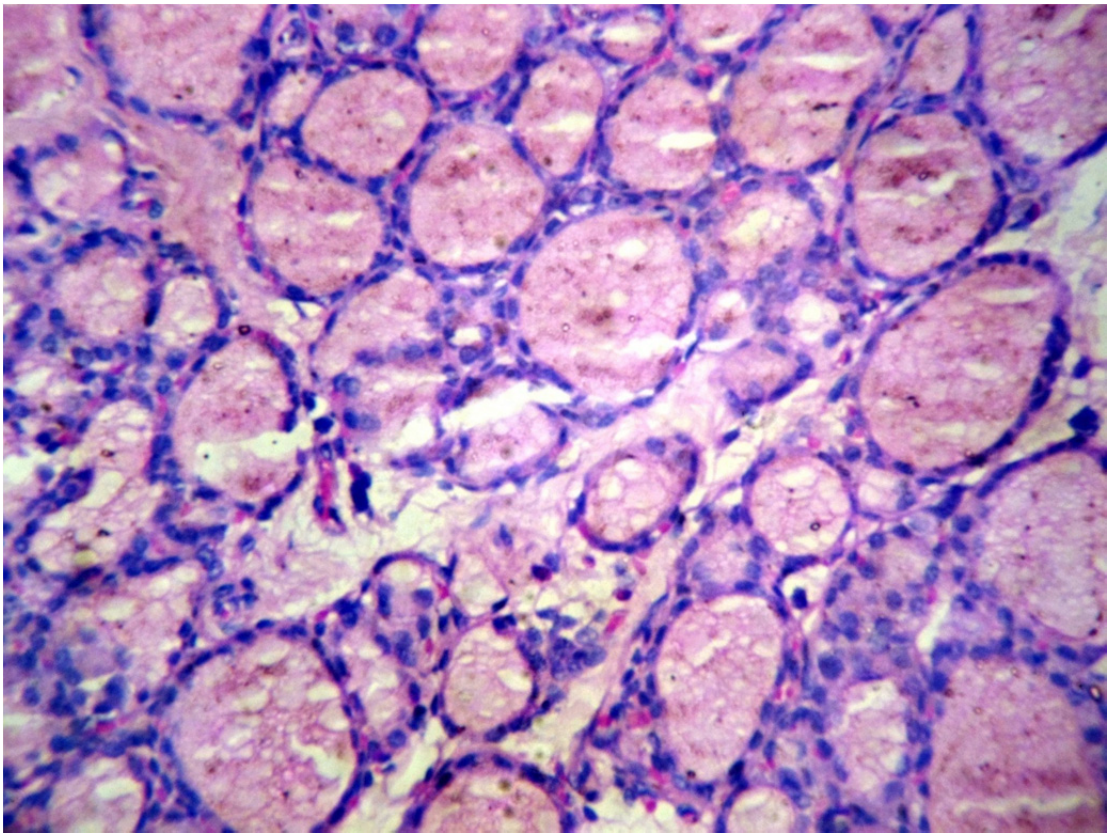
Thyroid gland section of GP2 rats (hyperthyroid control) showing follicles lined by cuboidal epithelial cells filled with 40-50% colloid.

FIG NO: 10



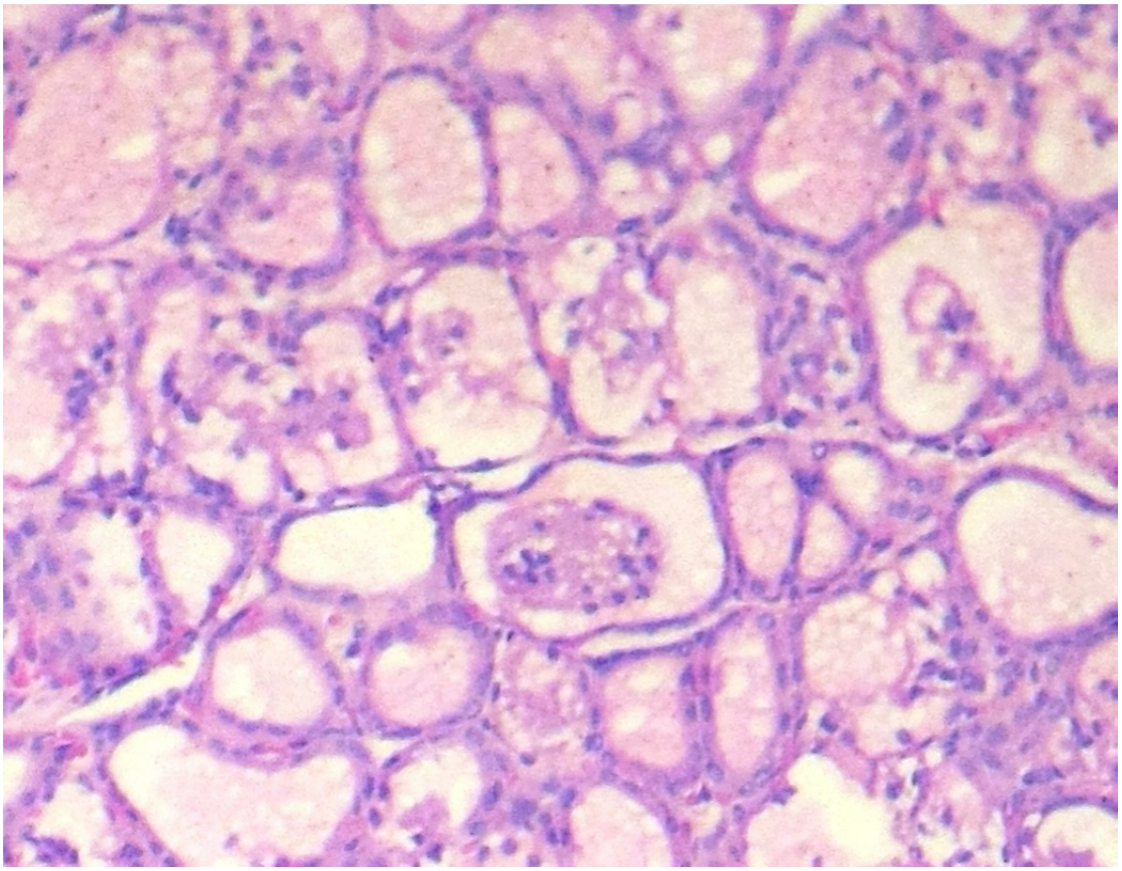
Thyroid gland section of GP3 rats (standard control at a dose of 10mg/kg) showing follicles lined by cuboidal epithelial cells filled with 80-90% colloid.

FIG NO: 11



Thyroid gland section of GP4 rats (HAEBD extract at a dose of 200mg/kg) showing follicles lined by cuboidal epithelial cells filled with 50-60% colloid.

FIG NO: 12



Thyroid gland section of GP5 (HAEBD extract at a dose of 400mg/kg/rat) rats showing follicles lined by cuboidal epithelial cells filled with 70-80% colloid.

RESULTS AND DISCUSSION

CHAPTER-8
RESULTS

❖ **Effects on the Serum Thyroid Hormones.**

L-THYROXINE (T_4) treatment induced significant ($P < 0.01$) increase of the serum T3 and T4 levels and decrease of the serum TSH contents. But 400 and 200mg/kg of BD extracts significantly ($P < 0.01$) and dose-dependently normalized the changes on the serum T_3, T4, and TSH concentrations induced by L-THYROXINE (T_4) as compared with L-THYROXINE (T_4) control. PTU 10 mg/kg also normalized the serum thyroid hormone levels, as similar as BD extracts 200 and 400 mg/kg, in the present study (Table 3).

❖ **Effects on the Serum AST and ALT**

Significant ($P <0.01$) increases of serum AST and ALT levels were detected in L-THYROXINE(T4) control rats as compared with intact control rats, controversially, AST and ALT concentrations in serum of PTU and both two different dosages of BD extracts treated rats were significantly ($P < 0.01$) decreased as compared with L-THYROXINE (T_4) control rats, respectively(Table 4).

❖ **Effects on the Liver LPO**

Continuous subcutaneous L-THYROXINE (T_4) injection induced significant ($P < 0.01$) increase of the liver LPO. But 400 and 200mg/kg of BD extracts significantly ($P < 0.01$) and dose-dependently normalized the changes on the liver LPO induced by L-THYROXINE(T_4) as compared with L-THYROXINE(T_4) control.PTU 10mg/kg also normalized the liver LPO comparable as BD extracts 200 and 400 mg/kg (Table 5).

❖ **Effects on the Liver Antioxidant Defense Systems**

In L-THYROXINE (T4) control, significant ($P < 0.01$) decreases of GSH contents were demonstrated with increases of SOD, and catalase activities as compared with intact control, respectively. However, both different dosages of BD extracts were dose dependently and significantly ($P < 0.01$) inhibited changes on the GSH, SOD and catalase. In addition, PTU also significantly ($P < 0.01$) inhibited the L-THYROXINE (T_4) treatment-related changes on the antioxidant defense systems as compared with L-THYROXINE (T_4) control (Table 5).

❖ **Effects on the Organ Histopathology**

In histomorphometrical analysis, significant ($P < 0.01$) decreases of the mean thicknesses of cross thyroid glands and follicular lining epithelium were detected in L-THYROXINE (T_4) control as compared with intact control. These L-THYROXINE (T_4) treatment related histopathological changes of thyroid gland, were dramatically inhibited by treatment of both dosages of BD extracts or PTU 10mg/kg (Fig8,9,10,11,12,).

DISCUSSION

Nowadays there is considerable interest in the potential health benefits of natural remedies such as medicinal plants and their extracts. One of these extracts is HAEBD which has different medicinal properties including anti-inflammatory, anticarcinogenic, platelet aggregation inhibiting, and metal chelating properties, etc [(124)] . The antioxidant effects of BD extract has been confirmed in different studies.[(125,126)] Some human clinical trials investigated various effects of BD[(127)] and there are some experimental studies about its antidiabetic effects [(128)] However, to date, there is no study on BD effects against hyperthyroidism. Therefore, in the present study, we investigated the effects of BD extracts on LT4-induced hyperthyroidisms and organ damages in comparison with those of PTU in rats with their possible antioxidant effects.

It has been believed that hyperthyroidism leads to oxidative damage of various organs and antioxidants have been reliable and favorable effects on hyperthyroidism[(129)].It is also expected that BD extracts also may be showed beneficial effects on hyperthyroidisms and related organ damages.LT4-induced hypothyroidism and related liver damages were normalized by 15 days continuous oral treatment of BD extracts 400 and 200mg/kg from 12 days after first LT4 treatment. Especially BD extracts enhanced the liver antioxidant defense systems—they dose-dependently inhibited LT4-induced increases of LPO and changes on the GSH contents, SOD, and catalase activities. These findings are considered as direct evidences that they have favorable ameliorating effect on the hyperthyroidisms and related organ damages induced by LT4 through antioxidant effects. The overall effects of BD extracts 400 and 200 mg/kg were similar to that of PTU 10 mg/kg, in the present study.

Thyroid hormones (T_3 and T_4) are involved in the -regulation of numerous body functions including lipid and carbohydrate metabolism, oxygen consumption, and several physiological functions such as development, reproduction and growth .[130] Alterations in their normal levels cause some biochemical and clinical abnormalities such as hypothyroidism and hyperthyroidism [131]. Extended exposure to the treatment with exogenous LT4 may alter thyroid activity by interfering with thyroid hormones synthesis, which provokes the disruption of thyroid axis, resulting in numerous abnormalities [132] Hyperthyroidism simply defined as increases of serum T_3 and T_4 with decrease of serum TSH, a pituitary hormone that regulated thyroid functions .[133,134] In the present study, LT_4-induced increases of serum T3 and T4 levels, and decreases of serum TSH concentrations were significantly and dose-dependently inhibited by treatment of BD extracts. In addition, BD extracts significantly ($P < 0.01$) inhibited the LT4-induced histopathological changes on the thyroid glands, the atrophic changes including decreases of mean thicknesses of follicular lining epithelium. These results are considered as direct evidences that GS extracts controlled the hyperthyroid states.400 and 200mg/kg of BD extracts showed comparable effects as compared with PTU 10mg/kg in this study.

Liver is a major target organ for thyroid hormone with important biological and medical implications[135], and serious liver damages accompanied to the thyroid hormone imbalances regardless of hyperthyroidism or hypothyroidism [136]. Clinical diagnosis of disease and damage to the structural integrity of liver is commonly assessed by monitoring the status of serum AST and ALT activities [137]. Higher activities of these enzymes in serum have been found in response to oxidative stress induced by hyperthyroidism [138]. Administration of BD extracts to rats resulted in inhibition of serum AST and ALT elevations. It has been well documented that thyroid dysfunctions increases LPO reactions and reactive oxygen species (ROS) [139]. LPO is an autocatalytic mechanism leading to oxidative destruction of cellular membranes[140]. Such destruction can lead to cell death and to the production of toxic and reactive aldehyde metabolites called free radicals, where MDA is the most important[141]. It is known that ROS would lead to oxidative damage of biological macromolecules, including lipids, proteins, and DNA [142,143] and oxidative stress also influenced to the body adipocyte results in decreases of body fat masses and related body weight decreases. MDA is a terminal product of LPO. So the content of MDA can be used to estimate the extent of LPO , and marked increases of liver MDA contents have been observed in hyperthyroid animals. GSH is representative endogenous antioxidants, prevent tissue

damage by keeping the ROS at low levels and at certain cellular concentrations,and accepted as protective antioxidant factors in tissues.

SOD is one of the antioxidant enzymes that contribute to enzymatic defense mechanisms, and catalase is an enzyme catalyzes the conversion of H_2O_2 to H_2O(155). The increase of some antioxidant enzymes activities such as SOD and catalase may be indicative of the failure of compensating the induced oxidative stress. In hyperthyroidism, it is well known that marked decreases of tissue GSH contents were induced, represent the decreases of antioxidant defense systems . Controversially, SOD and catalase activities were increase to remove over-produced ROS as of indication of the failure of compensating the induced oxidative stress. LT4-induced oxidative stresses and related organ damages were ameliorated by treatment of BD extracts in the present study like other previously tested antioxidants as direct evidenced that BD extracts have potent antioxidant effects enough to inhibited hyperthyroidisms. However, further mechanism studies should be conducted to clarify whether BD reduced oxidative damages of relative organs via improvement of thyroid function because dysfunction of thyroid hormones also can be lead to oxidative damages.

In the present study, we only focused on the *in vivo* protective effects to hyperthyroidism of crud extract itself not on the active compounds. Thus, these active compound searches should be proceeding in future.[144]

CONCLUSION

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CONCLUSION

In conclusion, LT4-induced hypothyroidism and related liver damages were inhibited by oral treatment of BD extracts 400and 200mg/kg. In addition, they also enhanced the liver antioxidant defense systems—they dose-dependently inhibited LT4-induced increases of LPO and changes on the GSH contents, SOD, and catalase activities as direct evidences that BD extracts have favorable ameliorating effect on the hyperthyroidisms and related organ damages induced by LT4 through antioxidant effects. BD extracts 400 and200 mg/kg showed comparable effects on the LT4-induced rat hyperthyroidism as compared with PTU 10mg/kg. These effects of BD may help the improvement of hyperthyroidisms and accompanied various organ damages, but active compound searches should be proceeding in future.

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CHAPTER-10

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